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ATABRINE STUDIES IN THE FIELD

I. The Relation of Serum Atabrine Level to Breakthrough of Previously Contracted Vivax Malaria[1]

LT. COL. ALEXANDER J. SCHAFFER, M.C. and CAPT. ROGER A. LEWIS, M.C.

INTRODUCTION

By mid 1943 two problems relative to the suppression of malaria demanded positive answers. First, was long continued atabrine administration efficacious; second, was it safe? Many of us who had used the drug freely in the South Pacific Area during the preceding year were confident that it was both. Troops in the field, on the other hand, often following the lead of their medical and line officers, had arrived at the opposite conclusion. They doubted the drug's usefulness in preventing clinical malaria and feared its alleged toxic effects. The disease had taken a heavy toll in ineffectiveness, though not in mortality, of the divisions engaged in the Guadalcanal campaign and continued to harass those same divisions in the rear areas to which they were sent to recuperate and retrain. The "X" Division suffered attacks at the rate of 1000 to 2000 per 1000 per annum while exposed on Guadalcanal, at which time they were supposedly taking 0.4 gms. (4 tablets) of atabrine weekly. Instead of falling, their rate rose to 4000 per thousand per annum five months after their arrival on non-malarious Fiji, four months after suppressive treatment had been discontinued.

During the month of September 1943 a study of the efficacy of atabrine suppression was set up in one regiment of this division. Approximately 600 men were given 0.1 gm. of the drug three times a day for one week following which one-half of them received 0.1 gm. daily, the other half 0.05 gm. dai y. 600 others did not receive the drug and constituted the control group. In the four weeks of observation 157 controls, but only 14 of the 600 who were presumably under suppressive therapy, developed malaria. Atabrine levels in the blood serum of the 14 who "broke through" were consistently lower than those who did not. There were too few breakthroughs for final conclusions to be

[1] This work was done in February–May 1944.

drawn as to this relationship. Nevertheless, it was clear that under the conditions of this experiment atabrine was capable of reducing vivax recurrences to one-tenth their previous rate. An answer was wanted as to why even 14 men had broken through suppression.

In order to test these questions more conclusively the authors and an enlisted technician were detached from the 18th General Hospital to carry out a similar but larger study on a division returning to New Zealand to rest. The object of the investigation, as laid down in a memorandum signed by Col. B. M. Baker, Medical Consultant, U.S.A.F.I.S.P.A., was to determine whether malaria broke through because of—

a) Insufficient atabrine to produce or maintain a level which protects, or—
b) The acquisition by the parasite of resistance to atabrine, or—
c) Increasing laxity in taking atabrine.

The subject was "Y" Division. One of its three infantry regiments had been heavily exposed to malaria for 16 months, the other two moderately exposed for 12 months. The former had been receiving suppressive atabrine for 14 months, the latter two for 12 months. All had supposedly taken 0.4 gm. weekly through December 1943; in January and February 1944, to the start of this experiment, they were offered 0.6 gm. weekly. Approximately 35% of all the men had had malaria at least once.

METHODS

Three companies (404) men) were taken off atabrine suppression to serve as controls and to supply a further indication of the degree of malarial seeding which had taken place in the division. Serum atabrine levels were done on all these men promptly upon their arrival in New Zealand and just prior to cessation of suppression. In addition, preliminary levels were performed upon each man in six other companies, two companies from each of the infantry regiments. These men were left on the suppressive treatment of 1 tablet (0.1 gm.) six times weekly, as was the remainder of the division.

Thereafter, every man from the division suspected of having malaria was admitted to the 39th General Hospital and his serum atabrine level determined. Malaria was not diagnosed or treated without

confirmation by positive smear. The levels on the positive smear malarias were considered "breakthrough levels," those on men who proved to have other diseases were utilized as additional controls.

Every individual from whom blood was withdrawn for level determination was questioned at length. He was assured that information obtained would be treated as confidential. All the facts pertinent to his malaria exposure and most important of all, his degree of faithfulness in taking suppressive atabrine, were noted upon an individual card. With the proper approach the truth could be elicited from a surprisingly large percentage. Every effort was made to be certain that the soldier had not taken any atabrine, other than his daily suppressive tablet, between the onset of the illness and the time he reported to the hospital. Self-treatment with atabrine for any febrile and some afebrile indispositions was fairly common practice among these veterans of the Solomon Islands campaigns. If additional atabrine had been taken or if it was suspected that additional atabrine might have been taken the soldier was excluded from the series. The depth of pigmentation was estimated at the time blood was withdrawn, the soldier being given a rating of 0, light, moderate, or heavy on the basis of the visible coloration of the skin of the hands, face, neck and chest.

Serum atabrine level was determined by the method evolved for use in the field by one of us (R. A. L.). Briefly, it is done as follows. 12–15 cc. of blood was taken before breakfast, and allowed to clot in a 15 cc. centrifuge tube, then ringed and centrifuged. The supernatant serum was transferred to a Wassermann tube and recentrifuged. 5 cc. of this supernatant was placed in a 15 cc. centrifuge tube, 1 cc. of normal sodium hydroxide and 5 cc. of petroleum ether added. The tubes were stoppered, shaken vigorously 5 times, and centrifuged for 5 minutes. A few drops of absolute alcohol were added, the coagulum stirred and the tubes recentrifuged. 4 cc. of the ether extract was placed in another 15 cc. centrifuge tube and shaken with 1cc. of saturated aqueous solution of chloral hydrate. The tubes were again centrifuged, the petroleum ether suctioned off and the tubes recentrifuged.

Standards made up to a concentration of .0005, .001, .002, .003, .005, and .010 mgms. % of atabrine dihydrochloride in serum or water

were treated in a manner identical with the unknown sera. The chloral hydrate extracts were compared with the standards in ultraviolet light in a dark room, using the same centrifuge tubes in which the extraction was made. Less green fluorescence than that manifested by the lowest standard was arbitrarily termed "none."

Glassware, corks and all other equipment used had to be meticulously cleaned or fluorescent contaminants were introduced. The ingestion of fatty food a few hours prior to withdrawal of blood tended to impart a grayish blue tint to the serum extract, rendering comparison with standards impossible. Quinine ingestion as much as 3 or 4 days before the test imparted to the extract a brilliant blue fluorescence completely hiding that of atabrine. Hemolysis of red cells, because of their higher content of the drug, made the serum reading incorrectly high.

RESULTS

A. PRELIMINARY LEVELS

Serum atabrine levels were determined upon 1021 men shortly after their arrival in New Zealand. This represented a very large sample of the entire division and was a fair indication of what might be expected of the other divisions in the South Pacific. The results follow in tabular form (Table I).

The arithmetic mean, or the approximate average, of all the levels was 13 micrograms per liter. 26% of the levels lie below 10, 64% below 20 micrograms per liter. The mode is 10 and the median 9. The standard deviation is 9. Approximately 67% of the levels lie between 4 and 22. (See Graph I)

The most striking phenomenon in this distribution is the extraordinary variation in levels achieved by individuals presumably on the same dosage of atabrine for the same period of time. This scattering had been observed by us on therapeutic schedules also.

The next significant finding became manifest when the figures were tabulated by companies. These follow (Table II).

It will be seen that companies differed significantly one from another. Their average levels varied from 9 to 20 micrograms per liter. Co. F contained no men with levels of 0 whereas 9% of Co. I had no demonstrable atabrine in their sera. Co. E had only 5% of men with concentrations below 10 micrograms per liter whereas Co. I had 47%

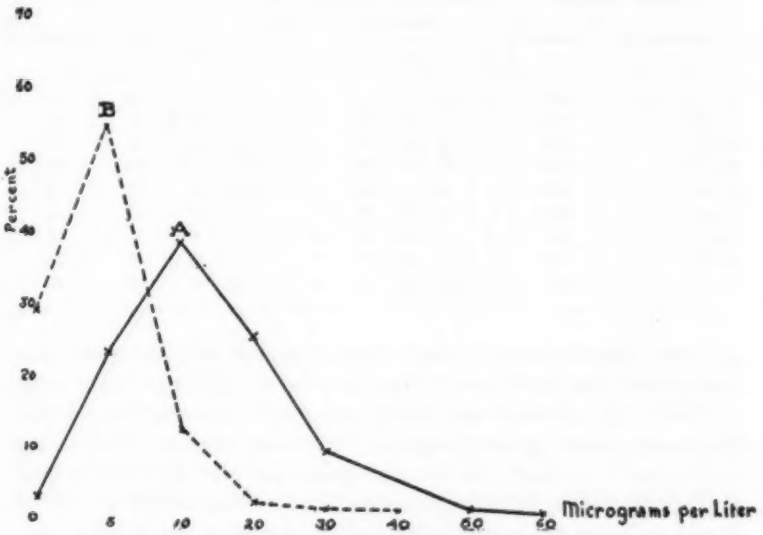
of such low level individuals. 6% of the former company and only 1% of the latter fell into the high level group, above 30 micrograms per liter.

TABLE I

*Distribution of Serum Atabrine Levels in Troops Supposedly on Suppression (0.6 gms. Weekly) in Micrograms per liter**

TOTAL.....	0	5	10	20	30	40	50	60
1021	39	228	390	253	89	16	5	1
100%	4	22	38	25	9	1.5	0.5	0.1

* Since readings were made by comparison with the nearest standard it will be seen that "5" may mean any figure between 3 and 8, 10 any figure between 8 and 13, and so on.



GRAPH I. PERCENTAGE DISTRIBUTION OF SERUM-ATABRINE-LEVELS

A—In 1021 men suppressed with 0.6 gms. weekly.
B—In 410 Breakthroughs.

One explanation of some of these apparent discrepancies can be put forward with assurance. One company differed from another in

average atabrine level and in percentage distribution because of differences in the strictness of the supervision of their atabrine distribution. To put it baldly, the commanding officers of some units saw to it that all their men received one tablet of atabrine daily, some did not.

Proofs for this blunt assertion are available. In the first place it seems highly unlikely that comparable groups of approximately 100 men could contain such widely divergent percentages of low level and high level individuals. In the second place the soldiers' own stories as to how faithfully they took atabrine bore it out. The average level of those who admitted they took the drug poorly was low and their

TABLE II

Atabrine Levels in Micrograms per liter in Various Companies Supposedly on the Same Suppression (0.6 gms. weekly)

ORGANIZATION	NUMBER	PERCENTAGE DISTRIBUTION								MEAN	% BELOW 10
		0	5	10	20	30	40	50	60		
Co. A	109	6	28	39	23	4	0	0	0	11	35
Co. B	83	5	32	40	18	4	1	0	0	11	37
Co. C	111	5	25	42	21	7	0	0	0	12	30
Co. D	120	4	30	38	18	7	2	1	0	12	34
Co. E	115	1	4	24	46	19	3	3	0	20	5
Co. F	130	0	14	44	29	12	1	0	0	15	14
Co. G	124	2	16	36	27	13	4	1	1	16	18
Co. H	112	3	17	44	28	7	1	0	0	13	20
Co. I	117	9	38	38	10	4	1	0	0	9	47

previous malaria incidence high when compared with the group who maintained they had missed little or none of the suppressive drug.

Finally, the question was readily susceptible to experimental test. Co. B was chosen as the test group, its average atabrine level having been found to be fairly low (11 micrograms per liter) and its low level individuals (below 10 micrograms per liter) to number 38%. On 19 March an order was issued at our request, signed by the division commander, enjoining perfect atabrine discipline upon the officers and men of this company. Levels were determined at the beginning of the program and at its end, four weeks later. The figures follow for the 58 men who were available for level determination *at both times* (Table III). The results are characteristic of the response of the entire company.

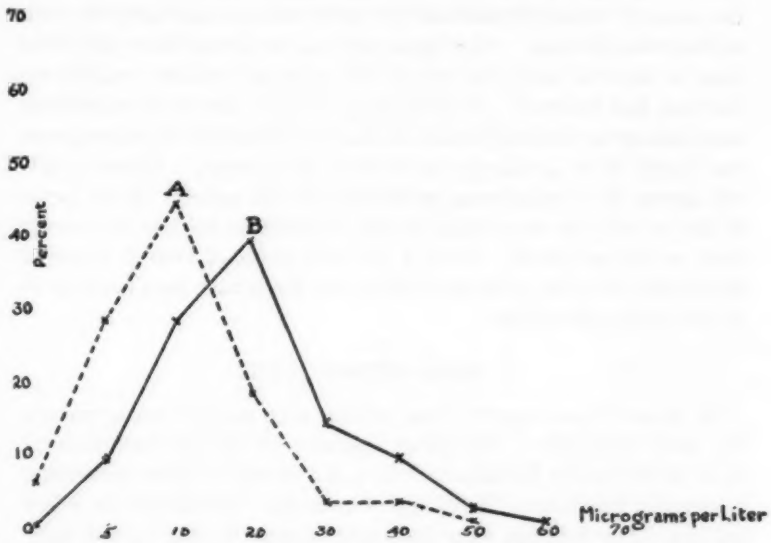
It will be seen that the institution of rigid discipline altered the picture significantly. All the "0" levels were eliminated. Levels

TABLE III

Atabrine Levels Before and After Four Weeks of Rigid Supervision in Soldiers taking 0.6 gms. weekly

Levels in Micrograms per Liter

	NUMBER	0	5	10	20	30	40	50	MEAN	% BELOW 10
Before	58	3	16	25	10	2	2	0	12	33
After	58	0	5	16	23	8	5	1	20	9



GRAPH II. PERCENTAGE DISTRIBUTION OF SERUM-ATABRINE-LEVELS IN A SUPPRESSED COMPANY

A—Before institutions of rigid discipline.
B—Four weeks after rigid supervision began.

below 10 were reduced from 33% to 9%. There was a corresponding increase in the number of men with levels of 20 to 50. The average rose in the month from 12 to 20 micrograms per liter (See Graph II).

Thus the reason for variability between company and company seems quite clear. This is not so for variation between individual and individual. Improvement in supervision raises the general level of a group's atabrinization but, as is amply demonstrated by Graph II, does not alter the shape of the curve appreciably. It appears undeniable that on the same dose of atabrine some men cannot achieve a level as high as 10 micrograms per liter whereas many attain 30 and an occasional man reaches 60 micrograms per liter. There is a rare soldier in whose blood serum no drug can be demonstrated by this method after months of suppression and who after large therapeutic doses also has "0" or only a "trace" in his serum. We have seen several such in the course of this study and had previously encountered seven in work with another division. All of these men had swallowed their prescribed doses of the drug under the eyes of one of us and neither vomiting nor diarrhea had followed. A small group of these low-level individuals were then given the drug intramuscularly and their blood level response was found to be strikingly lower than the average. (These results will appear in a forthcoming publication in this series). What factor or factors may be responsible for this inability to achieve an average level we do not know. From a practical point of view it is not of importance since the great majority of low levels have been found to be be due to poor discipline.

B. BREAKTHROUGH LEVELS

Of the 463 blood samples from soldiers with positive smear malaria 410 were acceptable. The prior ingestion of quinine had rendered most of the others unreadable. In a few instances other fluorescent substances interfered. Breakage of glassware accounted for a few failures. Ten readings were discarded because routine careful questioning brought out that extra atabrine had been taken by the soldier before admission to the hospital.

The levels at which malaria broke through follows (Table IV).

97.4% of the men who developed positive smear malaria had levels of 10 micrograms per liter or less. The arithmetic mean was 5, the mode 5, the median 5. The standard deviation is 4 and the 67 percentile range begins at 0 and ends at 9. (See Graph I).

Ten men only, therefore, broke through when the atabrine level was 20 micrograms per liter or higher. Four of these men were left untreated in order to follow their subsequent courses. One, with a breakthrough level of 20, ran a typical course with tertian chills. Another, with a level of 30, had no further fever or chills, his smears became negative, but he returned to hospital two weeks later with typical malaria. A third, level 20, had no chills after his original one for 10 days, when fever, positive smear and chills recurred. The fourth, admitted because of 1 chill, positive smear and with a level of 20, has had no subsequent chills for three weeks. It appears, therefore, that the breakthrough level may at times be high enough to be a therapeutic level. This paradox will be discussed later.

TABLE IV

Distribution of Breakthrough Levels

Levels in Micrograms per Liter

LEVEL	NUMBER	PER CENT
0	122	29.6
5	222	54.1
10	56	13.7
20	8	2.0
30	1	0.3
40	1	0.3
	410	100.0%

At this point it might be well to summarize, for comparison and contrast, several distribution patterns. Below are tabulated the atabrine level data for the highest level company in the study, for the 1000 man sample of the entire division, for the control group with fever of unknown origin or other febrile illness, and for positive smear malaria (Table V).

These figures require little elaboration. They indicate clearly what a high average level can be obtained with excellent discipline, what average atabrine level will be obtained by a division with only fair atabrine discipline and a malaria rate of about 250/1000/annum, and and to what a remarkable degree breakthrough is associated with depressed atabrine level. They indicate just as clearly that the

atabrine level is not depressed by febrile disease per se. (See Graph III).

In the three control companies taken off suppression, malaria developed throughout the three month observation period. It seemed of interest to determine whether those whose levels were high at the outset developed the disease later than those whose levels were low. Accordingly, the interval between cessation of suppression and first attack was plotted against the original level. The results follow (Table VI).

The conclusion seems clear even from such a small group that the higher the level the less likelihood there is of malaria occuring within 10 days and the greater the probability of its being posponed beyond 20 days. This point we feel is of great practical importance in that it demonstrates that a high level confers a degree of reserve protection

TABLE V

Summary of Data on Atabrine Levels

Levels in Micrograms per Liter

	NUMBER	ARITH. MEAN	% BELOW 10	% BELOW 20
Co. E	115	20	5	29
Division Y sample	1021	13	26	64
Febrile Illnesses other than malaria	141	12	43	66
Malaria	410	5	84	97

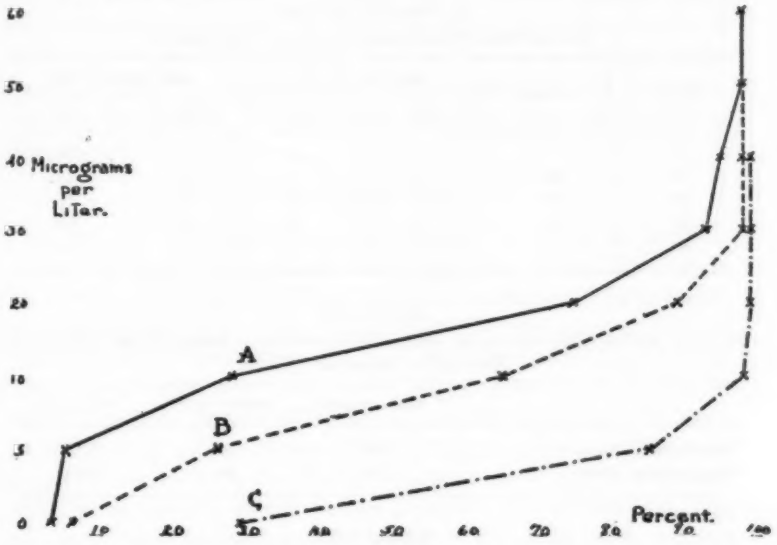
which a low level does not afford. If under combat conditions atabrine cannot be supplied, high level individuals will be protected for longer periods than others. The same may be true for other precipitating factors such as fatigue or alcoholism whose mode of action is at present unclear.

It is obvious from the foregoing that although breakthrough occurs most commonly when the atabrine level is below 10, it also occurs not too infrequently at 10, and rarely even when it is as high as 20, 30 or 40 micrograms per liter. Thus there is no set "protective level." One is only justified in saying that at a level of 10 the majority of seeded individuals appear to be protected from recurrence and that at a level of 20 almost all are so protected. This matter too will be discussed more fully later.

C. MALARIA RATES

A study of the case incidence throughout the observation period discloses several items of interest.

1. The three unsuppressed companies developed malaria between six and seven times more often than the suppressed. The figures follow (Table VII).



GRAPH III. CUMULATIVE PERCENTILE GRAPHS OF SERUM-ATABRINE LEVEL DISTRIBUTION

A—In Company with excellent discipline.
B—In 1021 man sample of a division.
C—In 410 Breakthroughs of malaria.

2. In the three unsuppressed companies followed eight, nine and ten weeks respectively after the cessation of suppression the case incidence appeared to be governed by the preliminary level. This astonishing result may indicate that good suppression continued over a long period actually prevents the development of some vivax malaria as it is now known to prevent the falciparum form. It may, on the other hand,

only mean that companies with good atabrine discipline also exercised excellent malaria control in all its other aspects. The tabulation below is, at any rate, provocative (Table VIII).

TABLE VI

Interval between Cessation of Suppression and Development of Malaria in Relation to the Original Atabrine Level

In Micrograms per Liter

Time of Breakthrough after Cessation of Suppression

LEVEL	NUMBER	LESS THAN 10 DAYS		MORE THAN 20 DAYS	
		No.	%	No.	%
0	2	1	50	1	50
5	26	2	8	16	60
10	27	0	0	21	78
20	11	0	0	10	90
30	3	0	0	3	100

TABLE VII

Cases of Malaria/1000/Annum in Companies Supposed to be taking 0.6 gms. Weekly vs. Those on No Suppressive Therapy

	TOTAL STRENGTH	NUMBER CASES	RATE/M/A
Suppressed Group	935	35	212
Unsuppressed Group	404	94	1340

TABLE VIII

Case Incidence in Companies after Cessation of Suppressive Therapy in Relation to the Preliminary Atabrine Level

	% PREVIOUS MALARIA	AVERAGE LEVEL	% BELOW 10 MICROGRAMS	CASES PER WEEK	RATE/M/ ANNUM
Co. F	34	15	14	1.8	559
Co. C	35	12	30	4.0	1793
Co. I	36	9	47	4.8	2025

3. Of the six companies presumed to be taking 6 tablets weekly, only one ended its period of observation, nine weeks, with the extraordinary record of not having had one breakthrough. This company had the highest average level (20) and the fewest low level individuals (5%)

of all the companies studied. The other five companies with average levels varying from 11 to 16 micrograms per liter broke through at rates which are not significantly different, between 160 and 370 per 1000 per annum.

4. The weekly rates in Co. B are of great interest. This is the unit which was the subject of the discipline experiment described above. For four weeks its average preliminary level of 11 was operative, during the succeeding four weeks of rigid supervision the level arose to 20 where it presumably remained for the final two weeks of obxervation. During the first four weeks six cases broke through (rate 404/thousand/annum); in the last six weeks three recurred (rate 154/thousand/annum).

D. PIGMENTATION

An attempt was made to correlate the depth of yellowish discoloration of the skin with the concentration of atabrine in the blood serum. This was found to be not possible in many instances. This is understandable inasmuch as there is a lag of several weeks between rise and fall of blood level and appearance and disappearance of pigmentation. Thus three weeks after cessation of suppression the level may be nearly 0 whereas pigmentation may persist for eight weeks or more. In the case of groups of men, however, since yellowness is *usually* proportionate to level, the degree of atabrinization can be estimated from the appearance of the men as a group. Our ratings of a large number of men examined consecutively and their actual levels compare as follows (Table IX).

This degree of correlation makes it possible to estimate quickly and with a fair degree of accuracy the general level of a unit's atabrine discipline.

COMMENTS

Little more need be said concerning Captain R. A. Lewis' method than has appeared in previous paragraphs of this report. In over 1600 determinations there was only one inexplicably high aberrant. The numerous prepared controls were almost invariably read correctly. Readings made independently by two, sometimes three observers, were usually identical although rarely what was called "trace" by one was

called "0" by another or an occasional high "trace" was read as "10" by the other. This individual variation is of the order expected when two men make colorimetric hemoglobin readings on the same blood, and the discrepancies are not significant. Two technicians easily completed the venapunctures, serum separation, and extraction of 60 bloods daily by 3 or 4 P.M. and the visual reading of this number of levels took at the most 15 minutes. The ready recognition of quinine lends the method additional virtue.

As previously pointed out the "breakthrough level" is not a fixed point on the scale of atabrine concentrations. 85% of breakthroughs take place when the level is 0 or trace, over 10% when the level is 10 micrograms per liter, the remainder at higher levels. An explanation for this phenomenon can be arrived at only if one considers the problem

TABLE IX

Depth of Pigmentation and Atabrine Level Correlation (880 men)

LEVEL IN MICROGRAMS PER LITER	VERY LIGHT	MODERATE	DEEP
0–10	61	26	13
10–20	34	35	31
20–30	11	36	53
over 30	0	12	88

of development of breakthrough as a dynamic one and the result dependent upon the interplay of three forces. In one direction the reproductive force of the plasmodia is constantly at work throwing parasites into the circulating blood. Unopposed this brings about parasitization of enough red cells to precipitate clinical malaria. In the natural state the opposing force is the sum of the immunological defense mechanisms. It has been shown that certain antibodies are at a minimum before and at the onset of an attack and that their titer rises as the disease progresses only to fall again after the lapse of weeks or months. Thus this force is cyclic, of sufficient strength at times to kill all or almost all the newly formed parasites but at other times powerless to protect from the disease.

In atabrine suppression another antagonistic force is added to the natural one. A low concentration of the drug may be sufficient to kill

off the few parasites that evade the biological barrier when that barrier is fairly efficient. When it is at its lowest ebb, however, plasmodia may irrupt into the blood stream in such quantity that a higher level of atabrine may be required to prevent clinical symptoms. Indeed it is conceivable that protection might not be afforded no matter how high the level, given a tremendous invasion over a short period of time, although the same level will be curative if maintained for a day or two. This mechanism must be operative in those men under suppression who experience an isolated chill.

Precipitating factors such as chilling, over-fatigue, over-indulgence in alcohol, etc., operate toward increasing the quantitative blood stream invasion. Whether this effect is due to lowering of the natural defense barrier or to enhancement of the plasmodium's power to multiply is not known and in this connection is irrelevant.

By the same token the "protective level" cannot be assigned a fixed value. Most individuals under ordinary conditions will undoubtedly be protected from recurrence with a level of 10; some will recur in spite of levels of 20 micrograms per liter or more. For a group of men, however, an average level of 20 confers almost perfect protection whereas average levels of 11 to 16 permit a breakthrough rate of from 200 to 500 per thousand per annum. Below an average level of 10 the rate increases rapidly. A level of 20 was attained by Co. E with their usual method of atabrine distribution and supervision; Co. B arrived at that average level after correcting its supervisory tactics. It, therefore, can be achieved and must be the mark toward which units aim.

Stated in other words a low-level index (% of levels less than 10 micrograms per liter) of less than 10% gives a unit excellent protection. Owing to the tremendous variability in atabrine utilization all men in a given unit cannot achieve levels of 10 or more on the usual suppressive dosage. This study indicates that the use of dosages above 0.7 gm. per week with the purpose of assuring each soldier such a level would be wasteful and unnecessary inasmuch as the breakthrough rate in the presence of a low-level index of 5 to 10 is so low as to be almost negligible.

It is not a certainty either that every man can achieve a level of 10 no matter how large the suppressive dose. It would seem that a few,

probably 1 in a thousand or less, are so constituted that atabrine never appears in the blood serum in this concentration, except perhaps fleetingly. (This exception is made because atabrine has always been effective in terminating chills even in those few individuals in whom no trace of the drug could be demonstrated in the blood 12 hours after doses as large as 1.5 gms. in 16 hours). The number of these poor utilizers of atabrine is so small that no effort need be made to discover them in advance. They will invariably, one would expect, become chronic recurrers, with short intervals between relapses, and at that point can be studied and excluded from malarious ones.

An average blood concentration of 20 micrograms per liter not only affords nearly perfect protection but provides an ample reserve for unforeseen contingencies. Should atabrine become unavailable to an outfit far removed from its base of supply, or isolated in the course of combat, a full week would elapse before the average level would drop to 10, hence protection would be good for at least that period. In the same fashion precipitating factors such as over-fatigue, prolonged wetting and chilling, etc., might be expected to cause little or no break-through in a highly atabrinized unit.

In order to keep a unit's low-level index down commanders must be on the lookout for all possible loopholes. Rosters must be complete and officers and non-coms entrusted with the daily check must be made thoroughly aware of the importance of their task. Men who do not appear regularly at formations or mess (cooks, M.P.'s, K.P.'s, buglers, those on detached service, etc.) must not be overlooked. Men on leave must be given additional atabrine before leaving and after return, or, better still, must be educated to the point of continuing their daily dose throughout their furlough period. Palming or discarding the drug after receiving it must be guarded against. Above all, the men should be instructed by pamphlet or by talks by the officers of the non-toxicity of the drug and of the need from the military standpoint and the advisability from the soldier's own standpoint of absolute faithfulness in atabrine medication.

These results apply only to recurrence of previously acquired vivax infection. Included in the results are only two quartan and four falciparum infections. It is likely that the results are applicable to fresh mosquito-borne vivax malaria, but one cannot be certain of this until studies are carried out in a malarious area. Here too it will soon

be seen whether they are applicable to falciparum disease also. (This study was later continued in a highly malarious area and the results reported to the Surgeon General. They will be submitted for publication shortly.)

SUMMARY

1. Atabrine concentrations were determined in the sera of 1021 members of a division which had been on suppressive treatment for 12 months or more. For the two months prior to this experiment they had been offered 0.6 gms. (six tablets) weekly; before that the prescribed dose had been 0.4 gms. weekly. The levels of 410 men who developed recurrent or delayed primary vivax malaria were also estimated, as were those of 141 men who came down with febrile illnesses other than malaria.

2. The average level of the division sample was found to be 13 micrograms per liter. The average level of men with illness other than malaria was 12 micrograms. The average malaria breakthrough level was 5 micrograms.

3. Companies were found to vary widely one from another in average level and in percent of low level individuals. This variation was proved to be due to differences in atabrine discipline. With excellent supervision an average company level of 20 micrograms per liter was achieved.

4. The malaria rates followed closely a unit's average level in inverse ratio, high level units having low rates and vice-versa.

5. The protective level and the breakthrough level were found not to be fixed points. Breakthroughs seldom occurred at levels as high as 20 micrograms per liter, very rarely at even higher levels. An explanation for this phenomenon was suggested.

6. *Recurrent vivax malaria breaks through suppression almost entirely because the atabrine concentration of the serum is low. The concentration is low, with few exceptions, because the drug has not been taken in prescribed dosage.*

The authors wish to acknowledge the help of Dr. Margaret Merrell, of the Department of Biostatistics, School of Hygiene and Public Health, who was good enough to review the statistics concerned in this report.

STUDIES ON CONVALESCENCE

IV. Nitrogen and Mineral Balances During Starvation and Graduated Feeding in Healthy Young Males at Bed Rest

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Earlier reports from this clinic have dealt with the large protein losses sustained by vigorous healthy males during convalescence from fracture of large bones of the lower extremity (1, 2). In agreement with Cuthbertson (3) it was concluded that disuse atrophy, bed rest, anesthesia, fever and infection per se do not account for the major portion of such protein wastage. Similar losses of protein have been observed after fracture by Browne, Stevenson and Schenker (4). The same pattern of increased protein breakdown and nitrogen wastage has been observed in patients suffering from burns (Browne) (5), in patients suffering from various types of infection, including meningitis and scarlet fever by Peters and coworkers (6) and in patients suffering with induced malaria (7).

In an attempt to evaluate what moiety of the lost nitrogen resulted from the altered protein metabolism incited by the trauma, an hypothetical curve was elaborated to portray the nitrogen balance that might be expected in a healthy individual at bed rest at various protein and caloric levels of intake (8). The variable factor of size was eliminated by introducing a correction factor based on the surface area of the subject, calculated from the height-weight tables of Dubois (9). All metabolic data were multiplied by

$$\frac{1.73}{\text{initial surface area of subject (in sq.m.)}};$$

and are thereby expressed in terms of an "average man," weighing 60 kg., 175 cm. in height (8).

The present communication deals with observations made on two

The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Johns Hopkins University.

healthy young male volunteers,[1] who were subjected to bed rest and diets which varied from complete starvation to one and one half times the calculated requirement. These studies were made to test the validity of the hypothetical nitrogen graph (8) and also to follow the total balance of potassium, phosphorus, sodium and chloride during brief periods of starvation and repletion in healthy subjects at bed rest.

Diets were prepared and given to the patients on the Metabolism Ward by the dietician in charge, Mrs. Elizabeth Bailey. Complete samples of all the diets were analyzed in the laboratory. Dietary changes were so arranged that, with each alteration, all of the constituents under study rose or fell proportionately—i.e., when dietary protein was raised by 25%, there was likewise a 25% increase of carbohydrate, fat, sodium, chloride, potassium, calcium and phosphorus. Some deviations from the exact desired intake were necessitated by the fact that the dietary components as calculated from the standard text used (10) were not always found to agree exactly with the laboratory analyses. The latter were, of course, not available until a later date. In the final tables the dietary figures are given both as calculated and analyzed for the sake of comparison; only analyzed data were used in the computation of the balances.

Water was allowed ad libitum. Urine collections were made directly into bottles containing 10 cc. of 25% acetic acid and 1 cc. alcoholic thymol 10%. Exact 24-hour collections were ended at 9:30 each morning. Stools were collected over two and five day periods; the beginning and ending of periods being marked by the appearance of ingested carmine. No drugs were administered throughout the experimental periods except fluid extract of cascara as a cathartic on rare occasions. The patients, who were exceedingly cooperative and intelligently interested in the study, remained quiet in bed reading or listening to the radio, except for a single daily period of 15 minutes when use of the lavatory was permitted for shaving, washing and the use of the commode.

ANALYTICAL METHODS

Urine: Total nitrogen was determined by a macro-Kjeldahl technique (11), alpha amino nitrogen by the method of Albanese and

[1] The subjects of this study were conscientious objectors who volunteered for assignment to this project.

Irby (12), calcium by the method described by the Harvard Fatigue Laboratory (13), phosphorus according to Fiske and Subbarrow (14) and chloride by modified Polhard, Harvey method (15).

Sodium and potassium were determined by the flame photometer (American Cyanamid Company) (16). Precautions and general methods outlined by Hald and Peters (17) were used with some modifications made in our own laboratory (18). Solutions of known sodium or potassium content were tested immediately before and immediately after each unknown, and were found to check exactly before any determination was considered valid.

Stools: Total feces, marked by oral ingestion of 0.3 gms. carmine administered on mornings of beginning and ending of each period, were transferred, as excreted, with water washings, to a suitable covered enameled container and stored in the ice box at 10–12°C. Storage of specimens was never more than 24 hours, whereupon the specimens were evaporated to dryness on the steam bath, subsequent fecal specimens of the period being added as collected. The total collection of the period was left on the steam bath until dry. Dryness is easily noted by the brittle dessicated appearance. The container was reweighed, the weight of the dry feces noted, and the material was pulverized through a small manual meat chopper. The ground material was screened through a 60-mesh screen. The screened material was thoroughly mixed, and a suitable aliquot of approximately 4 oz. was bottled for subsequent analyses.

1 gram of the dried pulverized stool was accurately weighed and ashed in a platinum crucible in an electric furnace at 450–500°C. The ash (after 8–16 hours) was dissolved in a minimum amount of 18% HCl (usually not more than 1 cc.) and taken up in water to 100 cc. Dilutions of this solution were used in the determination of sodium and potassium (flame photometer) and of calcium (13) and phosphorus (14).

Fecal chlorides: 5 grams of dried pulverized stool were weighed and transferred to a 50 cc. volumetric flask. 30 cc. distilled water was added, and the flask was shaken at intervals for several hours. The flask was then made up to volume, and the contents were allowed to leach overnight. The suspension was centrifuged and 5 cc. portions of the supernatant fluid were used for chloride determination by the urinary method (15).

Fecal nitrogen[2]*:* 0.5 gm. of dried pulverized stool was weighed in duplicate, and nitrogen was determined by macro-Kjeldahl technique (11).

Blood methods: Blood was drawn under oil from brachial veins, with minimal stasis, and in the fasting state. Analyses were made by the following methods:

Serum uric acid (19); hematocrit (20); serum protein (11); serum chloride (21); serum CO_2 (22); serum calcium (23); serum inorganic phosphorus (14); serum non-protein nitrogen (24).

PROCEDURE

Prior to their admission to the hospital both young men had been in a camp, vigorously employed at manual labor. Before beginning any metabolic studies, they were found to be normal to physical examination and by laboratory tests. The first *experimental* period was one of abrupt starvation followed by graduated feeding, designed to duplicate as closely as possible the pattern of voluntary dietary intake previously observed in healthy young males after acute injury such as fracture. There was a *preliminary* control period of 10 days, the last five of which were spent in bed, during which diets were fed containing approximately 10 gms. protein nitrogen and 1600 calories per 1.73 square meters of surface area (8). There followed a 2-day period of complete starvation; next, two days on which one-fourth the control diet was fed; then two days with one-half the control diet, and so forth by increases of one-fourth of the control diet in 2-day periods until one and one-half times the original control diet had been reached. We then allowed a holiday of five days, the men being allowed free choice of food which we did not analyze, and they were allowed several hours daily absence from the hospital for exercise and recreation. During this recess most of their lost weight was regained, as was also their general sense of fitness and well being.

A second experimental period was now begun, a reversal of the previous one in that the patients were fed during the control period of five days one and one-half times the calculated diet for caloric and nitrogen equilibrium at bed rest (i.e., 15 grams protein nitrogen and

[2] By use of the storage of feces in the ice box for not longer than 24 hours and continuous steam bath drying of feces for any total period, the formation of ammonia by bacterial decomposition is minimized.

2400 calories per 1.73 square meters surface area). Thereafter at two day intervals the dietary intake was reduced by a fixed moiety, so that in the six periods they received, in terms of the "normographic" diet $1\frac{1}{2}$, 1, $\frac{3}{4}$, $\frac{1}{2}$, $\frac{1}{4}$ and 0. Bed rest was begun on day 30 (See Charts I and II). Complete data on the metabolic elements under observation during the test periods are afforded in the tables at the end of the article. *All metabolic data in the charts and tables have been multiplied by the factor* $\frac{1.73}{\text{patient's surface area in sq. meters at onset of experiment}}$.

RESULTS

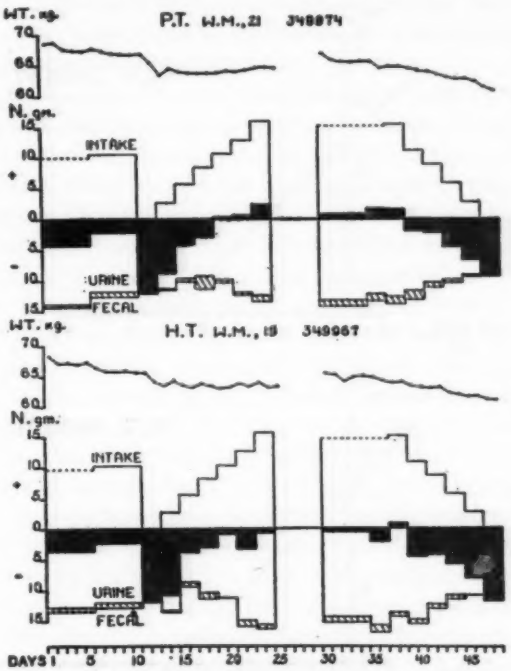
Behavior of nitrogen during period of abrupt starvation and graduated increased feeding. On Chart I is presented graphic representation of the balance data in respect to nitrogen. On days 1 through 5 and 30 through 36 dietary analyses were not made, the intake having been calculated from Sherman's tables (10) and the intake of nitrogen is therefore, denoted by an interrupted line for these days.

It may be seen that in the foreperiod of control (days 6 to 10), at bed rest, on diet containing approximately 10 grams nitrogen and 1600 calories per 1.73 square meters (so-called normographic diet), both patients were in negative nitrogen balance of slightly more than 2 gms per day. This had been expected from our previous experience with healthy vigorous persons at bed rest on such regimens (25). During the two days of total starvation both patients lost 12 gms. of nitrogen per day. As the dietary increments rose, less nitrogen was lost with each succeeding period. P.T. regained a small percentage of his lost nitrogen during the last six days of "normographic" and higher intake. H.T., however, who was the more nervous, high-strung and introspective of the two patients, did not come back into nitrogen balance at all during this experimental period, even during the 2-day period when fed one and one-half times the "normographic" diet.

When the data are plotted (black dots) on the hypothetical normograph (Chart II), it may be observed that during the two days of starvation, P.T. excreted 2 grams nitrogen more than predicted; but thereafter, with increasing diet up to normographic, the points fall within 1 gram of the "normographic" line.[3]

[3] It has been previously pointed out that, with diets increasingly greater than normal requirements, less and less of the excess nitrogen can be expected to be stored (8).

The other patient's (H.T.) curve of nitrogen balance is much less regular (Charts I and II). He, too, lost during starvation 2 more grams nitrogen per day than called for in the hypothetical graph. At

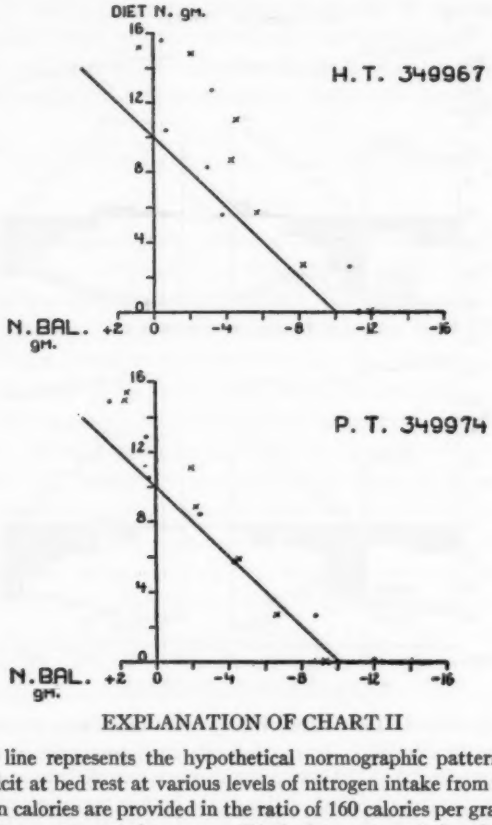


EXPLANATION OF CHART I

At the top is recorded the weight of the patient taken each morning on rising after emptying the bladder. The solid black areas denote the overall nitrogen balance in grams *per day*. The intake of nitrogen is recorded above, the output of nitrogen below; the cross-hatched portion at the bottom represents nitrogen in the stools. As mentioned in the texts, all metabolic data here recorded have been multiplied by a factor $\frac{1.73}{\text{initial surface area of the patient}}$. The blank area from day 25 to day 30 was the holiday period.

one-fourth intake he lost enough more nitrogen in the urine than at total starvation so as to conserve none of his ingested nitrogen, though in the succeeding 2-day period of one-half normographic feeding, he lost slightly less nitrogen than predicted.

During the period of starvation and increasing diet (days 11 to 25), patient P.T. lost weight from 66.9 Kg. to 64.7 Kg. Patient H.T. showed a similar weight loss from 65.5 Kg. to 63.1 Kg. (top of Chart I).



EXPLANATION OF CHART II

The solid line represents the hypothetical normographic pattern of expected nitrogen deficit at bed rest at various levels of nitrogen intake from 0 to 10 grams per day when calories are provided in the ratio of 160 calories per gram of nitrogen for each 1.73 sq. meters surface area. Black dots represent the nitrogen balance found on the two subjects here reported during the experimental period of abrupt starvation and graduated increased feeding. The points marked X represent nitrogen balances found on these same two subjects during the experimental period of graduated withdrawal of food.

In the holiday period (days 25 to 30) in which the patients were allowed to eat ad libitum on the ward or outside the hospital on some

occasions, weight was regained. P.T.'s weight rose from 64.7 Kg. to 67 Kg. (same as at onset of complete starvation), and H.T.'s weight rose from 63.1 Kg. to 65.3 Kg. (almost the same as at onset of complete starvation). How much nitrogen was restored during this period we do not know; but their intake was estimated at approximately one and one-half times the "normographic" diet.

During the first two days of total starvation, both patients were moderately annoyed by hunger; but thereafter neither experienced any noteworthy discomfort, though they were able to consume all the food offered without difficulty. On the first day of activity (day 25), both felt unsteady in the upright position and not anxious to move about much. Thereafter, however, they experienced no adverse symptoms and, so far as they could tell, felt just as well as usual.

Behavior of nitrogen during period of graduated reduced feeding to starvation. The two latter halves of Chart I depict the nitrogen balance during the second experimental period, in which observations were begun on diets containing one and one-half times the normographic constituents and then, at 2-day intervals, reduced to normographic, three-fourths, one-half, one-fourth and finally zero. In the fore-period (days 30 through 36), the interrupted line of nitrogen intake shows the diets to have been calculated and not analyzed through inadvertent failure of the diet to be sent from the ward. Analyses of the diets were begun with days 37 and 38; and, though from calculated figures we had expected this diet to contain approximately 12.5 gms. nitrogen, it was found on analysis to contain slightly more than 15 grams. Bed rest was resumed on day 30. It is of interest that patient H.T. was in positive nitrogen balance during days 37 and 38, the only time this was so during any of our experimental observations on him.

The pattern of nitrogen losses of the two patients during this experimental period is seen to assume almost a mirror image of the pattern during the experiment of abrupt starvation and graduated upward feedings. The losses of H.T. are seen to be greater than those of P.T. in the earlier periods (days 39 to 43). Thereafter, however, the nitrogen losses of the two men ran roughly parallel until, during the last two days total starvation, H.T. excreted 12 grams nitrogen per day, while P.T. excreted 9.5 grams nitrogen per day.

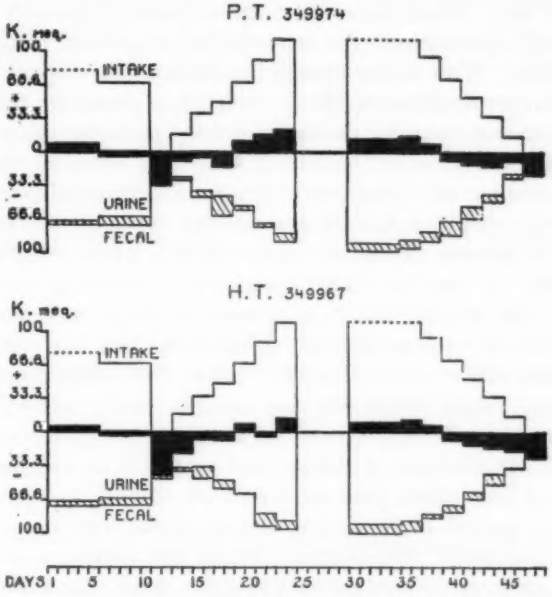
When charted against the normographic line (Chart II), the points

(marked X) in the case of P.T. fall almost identically on the line of his nitrogen curve in the preceding experiment (dots) in which abrupt starvation was followed by graduated increased feeding. And the same may be said for the plotting of H.T.'s data. However, at the lowest dietary intake and at starvation, the losses of nitrogen were slightly less than those observed at abrupt starvation and one-fourth diets. The preceding periods of relative starvation induced by the descending intake had doubtless brought about this metabolic alteration, in the same manner that "depleted" individuals have been found to be maintained in positive nitrogen balance on lower nitrogen and caloric diets than is possible with non-depleted individuals (2, 5).

Urinary Amino Acids. The urinary content of alpha amino nitrogen, determined by the method of Albanese and Irby (12), was followed daily throughout the experiments. The patients demonstrated a tendency to excrete less amino acid via the urine during their periods of undernutrition, both during the period of abrupt starvation and graduated increased feeding and during the period of graduated reduced feeding. Urinary amino acid output averaged around 175 mgm. per day when the patients were receiving diets greater than one-half the normographic calculation; and when diets below this were fed, the output averaged slightly over 100 mgm. per day. Determinations of amino acid concentration in the serum were not made.

Behavior of potassium during period of abrupt starvation and graduated increased feeding. On the first half of Chart III is represented the overall movement of potassium observed in the two men during the foreperiod at bed rest on normographic diet, and during the two day period of total starvation and graduated feedings. The first five days charted (dotted lines) may again be disregarded since the diets were not analyzed and the patients were not at bed rest. In the second five day period (days 6 through 10) on "normographic" diet, patient P.T. lost an average of 4 Meq. of potassium per day. In the two days of total starvation there was a loss of 33 Meq. potassium per day. When one compares these losses of potassium with those of nitrogen during the same periods (2 grams nitrogen per day and 12 grams nitrogen per day, respectively), it is seen that the losses of each substance are in approximately the same ratio as they exist in the intracellular compartment of muscle tissue (26) (2.5 Meq. potassium per gram nitrogen). We might visualize that the two substances have

been expended by the organism from the intracellular compartment together. However, in the next two 2-day periods with nitrogen losses of 9 and 4.5 grams per day, the potassium deficits were slightly less than 10 and 6 Meq. per day. With the next rise in diet (days 17–18), the deficit of nitrogen was 3 grams per day, deficit of potassium



EXPLANATION OF CHART III

Solid black areas denote the overall potassium balance. Intake and output are expressed above and below the zero line. Dotted lines indicate that dietary potassium was estimated, solid line that diet was analyzed. Both urinary and fecal potassium were analyzed throughout.

14 Meq. per day. In the following three 2-day periods with a total nitrogen repletion (positive balance) of 7 grams in the six days, there was a retention of potassium amounting to 100 Meq. We may say then that in this patient during two days of starvation and four days of low feeding, he lost nitrogen and potassium at approximately intracellular proportions; but that, when fed basally and more abun-

dantly in the next six days, potassium was repleted faster than was nitrogen.

In the case of the other patient, H.T., a very similar movement of potassium is seen to occur (Chart III). During starvation and one-fourth feeding periods, the patient exhibited losses of 12 and 11 grams nitrogen per day, and losses of potassium amounting to 45 and 21 Meq. per day. When these figures are averaged, it is found that the ratio closely approximates the intracellular proportions of these two constituents. With higher dietary increments this patient did not regain nitrogen equilibrium, though repletion of potassium was begun.

The behavior of potassium during the period of graduated reduced feeding to starvation. The latter portions of Chart III represent the movement of nitrogen and potassium during the experimental period when the patients were first fed one and one-half times the normographic diet and a stepwise *diminution* of the dietary intake was thereafter carried out. It may be recalled that, prior to beginning this series of test diets, the patients had been allowed five days' furlough, during which time they ate as desired. Presumably they ate hungrily for both gained weight (days 25 to 29). There then followed a period of seven days during which one and one-half times "normographic" diet was fed, but the diets were not analyzed and the patients were not at bed rest (thus intakes of nitrogen and potassium are represented by interrupted lines rather than solid lines for days 30 to 37). In the subsequent periods of descending dietary intake from one and one-half "normographic" diet to zero, nitrogen and potassium are lost in approximate proportion to their intracellular ratios, just as during the starvation periods previously described. Thus again, *in losses sustained during periods of underfeeding,* nitrogen and potassium were lost from the body in the same ratio that they normally exist in cells.

On Charts IV and V are represented only the *total* balance data for nitrogen and potassium during the experimental periods described above; the plotting is so designed that the same space represents 1 gram nitrogen as 2.5 Meq. potassium. According to the condition of these experiments, nitrogen and potassium are seen to move together from the intracellular compartment during "depletion phases," there being manifest a conspicuous tendency to restore potassium sooner than nitrogen during the "repletion phases."

Behavior of phosphorus during periods of abrupt starvation and

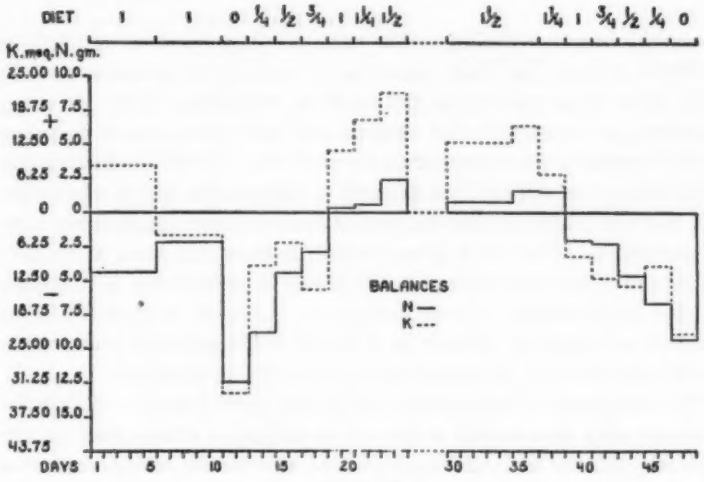


CHART IV

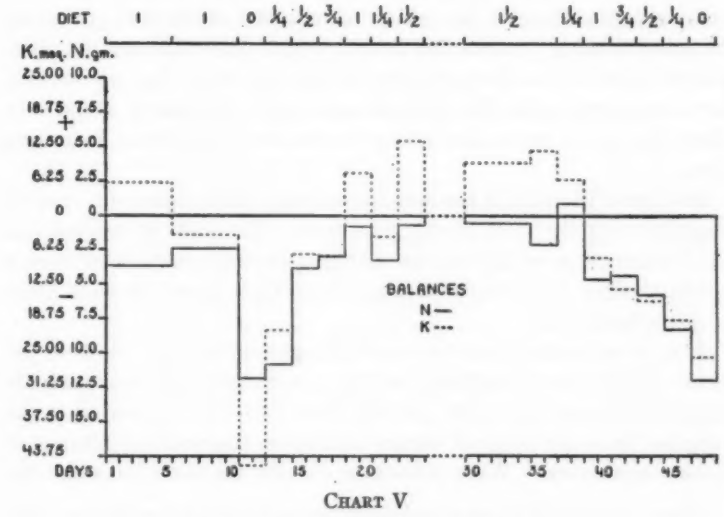


CHART V

EXPLANATION OF CHART IV AND V

Solid line indicated nitrogen balance, dotted line potassium balance. Days 1 through 5 and 30 through 36 intake was calculated, not analyzed. The chart is so arranged that 1 gram nitrogen is afforded the same spacing as 2.5 Meq. potassium (see text).

graduated increased feeding. The ratio of phosphorus to calcium in analyses of bone has been found to be reasonably constant (1:2.2) even in a wide variety of pathological conditions (27). It may, therefore, be construed that calcium and phosphorus move together in these proportions into and from the skeleton. If one knows then the total balance of calcium, the amount of phosphorus which was either laid down with this calcium in bone or removed from the skeleton may be estimated. Thus in a given period, if there has been a positive balance of 1 gram of calcium, 0.454 grams of phosphorus is conceded to have been utilized in bone formation. Likewise if there has been a deficit of 1 gram of calcium in a period, 0.454 grams of phosphorus has left the bones to be either excreted or utilized elsewhere.

The movement of phosphorus which has participated in metabolic processes *other than skeletal* is derived by adding or subtracting (as the case may be) the skeletal phosphorus to or from the *total* phosphorus balance.

In Tables 1 and 2 the intake and output of *total* phosphorus and calcium are given for the periods of abrupt starvation and graduated increased feeding. In the last column of the table is shown the phosphorus balance after correction for the amount which had presumably been concerned with the skeletal exchange. In Tables 3 and 4 is given the same type of data during the periods of graduated decreasing diets.

In Charts VI and VII the *non-skeletal* phosphorus balance is charted together with the total nitrogen balance. The scale for phosphorus is 15 times as great as that for nitrogen since in analyses of muscle approximately 15 grams of nitrogen have been found for each gram of phosphorus (28).

It is to be recalled that the total phosphorus balances in these patients include the phosphorus of the stools, and that inevitably in calculations over such short periods there must be error owing to the time lag from the point of dietary change to the resultant change in stool composition.[4] When allowance is made for these facts and the

[4] When one looks at the stool phosphorus and total phosphorus balances, large variations are obviously due to fecal lag. However, in all likelihood the same lag is affecting fecal calcium, and the non-skeletal phosphorus balance is thus not so subject to this source of error as might be thought at first glance.

TABLE 1

Total Calcium, Total Phosphorus and Non-skeletal Phosphorus Balances

P.T. J.H.H. #349974

A. Starvation and graduated increased feeding.

DAY	DIET	CALCIUM				PHOSPHORUS				NON-SKELETAL P (P-Ca/2.2)
		Intake	Urine	Stool	Bal.	Intake	Urine	Stool	Bal.	
6	1	970	137	932	−99	1221	883	453	−115	−70
7	1	970	137	932	−99	1221	883	453	−115	−70
8	1	970	137	932	−99	1221	883	453	−115	−70
9	1	970	137	932	−99	1221	883	453	−115	−70
10	1	970	137	932	−99	1221	883	453	−115	−70
11	0	0	140	266	−406	0	879	141	−1020	−835
12	0	0	140	266	−406	0	879	141	−1020	−835
13	1/4	232	171	266	−205	304	751	141	−588	−495
14	1/4	232	171	266	−205	304	751	141	−588	−495
15	1/2	475	151	499	−175	630	671	307	−348	−268
16	1/2	475	151	499	−175	630	671	307	−348	−268
17	3/4	776	132	2140	−1496	738	620	1151	−1033	−353
18	3/4	776	132	2140	−1496	738	620	1151	−1033	−353
19	1¼	970	152	409	+409	1221	783	211	+227	+41
20	1¼	970	152	409	+409	1221	783	211	+227	+41
21	1¼	1244	191	371	+682	1598	926	200	+472	+162
22	1¼	1244	191	371	+682	1598	926	200	+472	+162
23	1½	1398	173	1008	+217	1807	942	580	+285	+186
24	1½	1398	173	1008	+217	1807	942	580	+285	+186

TABLE 2

Total Calcium, Total Phosphorus and Non-skeletal Phosphorus Balances

H.T. J.H.H. #349967

A. Starvation and graduated increased feeding.

DAY	DIET	CALCIUM—MGM.				PHOSPHORUS—MGM.				NON-SKELETAL P (MGM.) (P-Ca/2.2)
		Intake	Urine	Stool	Bal.	Intake	Urine	Stool	Bal.	
6	1	954	176	882	−104	1201	1020	511	−330	−283
7	1	954	176	882	−104	1201	1020	511	−330	−283
8	1	954	176	882	−104	1201	1020	511	−330	−283
9	1	954	176	882	−104	1201	1020	511	−330	−283
10	1	954	176	882	−104	1201	1020	511	−330	−283
11	0	0	165	148	−313	0	844	103	−947	−805
12	0	0	165	148	−313	0	844	103	−947	−805
13	1/4	228	208	148	−128	299	882	103	−686	−628
14	1/4	228	208	148	−128	299	882	103	−686	−628
15	1/2	468	158	781	−471	619	712	533	−626	−412
16	1/2	468	158	781	−471	619	712	533	−626	−412
17	3/4	763	186	1094	−517	726	716	671	−661	−426
18	3/4	763	186	1094	−517	726	716	671	−661	−426
19	1	954	207	0	+747	1200	809	0	+391	+51
20	1	954	207	0	+747	1200	809	0	+391	+51
21	1¼	1223	265	1477	−519	1571	1032	954	−415	−179
22	1¼	1223	265	1477	−519	1571	1032	954	−415	−179
23	1½	1374	267	709	+398	1777	1028	452	+297	+116
24	1½	1374	267	709	+398	1777	1028	452	+297	+116

TABLE 3

Total Calcium, Total Phosphorus and Non-skeletal Phosphorus Balances

P.T. J.H.H. #349974

B. Graduated decreased feeding and starvation.

DAY	DIET	CALCIUM				PHOSPHORUS				NON-SKELETAL P (P-Ca/2.2)
		Intake	Urine	Stool	Bal.	Intake	Urine	Stool	Bal.	
35	1⅓	1382	204	1521	−343	1705	958	843	−96	+60
36	1⅓	1382	204	1521	−343	1705	958	843	−96	+60
37	1¼	1360	228	1503	−371	1731	880	861	−10	+159
38	1¼	1360	228	1503	−371	1731	880	861	−10	+159
39	1	1179	209	1541	−571	1200	946	880	−626	−366
40	1	1179	209	1541	−571	1200	946	880	−626	−366
41	3/4	791	194	661	−64	941	826	349	−234	−205
42	3/4	791	194	661	−64	941	826	349	−234	−205
43	1/2	548	180	296	+72	620	765	165	−310	−343
44	1/2	548	180	296	+72	620	765	165	−310	−343
45	1/4	253	181	201	−129	297	703	121	−527	−468
46	1/4	253	181	201	−129	297	703	121	−527	−468
47	0	0	212	0	−212	0	750	0	−750	−654
48	0	0	212	0	−212	0	750	0	−750	−654

TABLE 4

Total Calcium, Total Phosphorus and Non-skeletal Phosphorus Balances

H.T. J.H.H. #349967

B. Graduated decreased feeding to starvation.

DAY	DIET	CALCIUM—MGM.				PHOSPHORUS—MGM.				NON-SKELETAL P (P-Ca/2.2)
		Intake	Urine	Stool	Bal.	Intake	Urine	Stool	Bal.	
35	1⅓*	1359	315	1477	−433	1676	1110	963	−397	−200
36	1⅓*	1359	315	1477	−433	1676	1110	963	−397	−200
37	1¼	1337	313	827	+197	1702	1024	496	+182	+92
38	1¼	1337	313	827	+197	1702	1024	496	+182	+92
39	1	1159	329	827	+3	1180	1132	496	−448	−449
40	1	1159	329	827	+3	1180	1132	496	−448	−449
41	3/4	778	271	587	−80	926	827	354	−255	−219
42	3/4	778	271	587	−80	926	827	354	−255	−219
43	1/2	539	270	570	−301	610	756	435	−581	−444
44	1/2	539	270	570	−301	610	756	435	−581	−444
45	1/4	249	248	0	+1	292	769	0	−477	−477
46	1/4	249	248	0	+1	292	769	0	−477	−477
47	0	0	278	0	−278	0	946	0	−946	−820
48	0	0	278	0	−278	0	946	0	−946	−820

* Calculated diets.

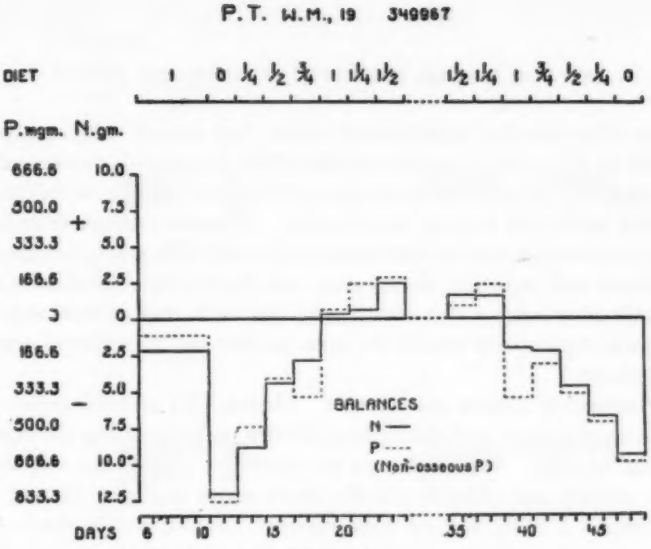


CHART VI

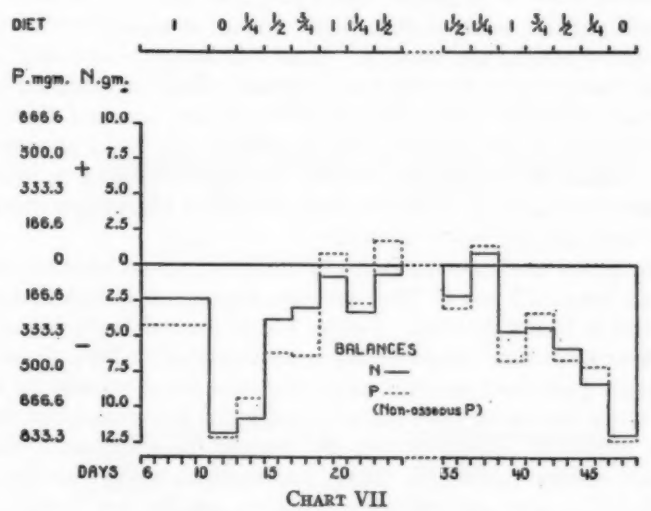


CHART VII

EXPLANATION OF CHARTS VI AND VII

The movement of phosphorus, after correction for the phosphorus presumed to have moved into or out of the bones (see text) is depicted by the dotted lines. Solid lines indicate the coincident balance of nitrogen. The scale used is such that 15 grams of nitrogen equals 1 gram of phosphorus.

many other smaller experimental errors that attend balance experiments of this kind, it seems amazing that the overall movement of nitrogen and nonskeletal phosphorus follow one another as faithfully as they have done in these experiments. It seems reasonable to infer that, under experimental conditions of this kind with graduated dietary depletion and repletion, *the nitrogen and phosphorus expenditures and deposits move together consistently, and that their proportionate expenditure and replacement remain the same as their ratios in normal muscle protoplasm.*

Movement of sodium and chloride. Charts VIII and IX depict the balances of sodium and chloride on the two patients during the experimental periods. Total balances are recorded, though the output of both sodium and chloride via the stools was a negligible factor. In one period 7 Meq. sodium were excreted per day in the stools, but this occurred during a preliminary period (days 30 to 35) during which diet was not analyzed. Otherwise there was never found more than 2 Meq. sodium per day in stools and usually less than 1 Meq. Chloride was never found to be greater than 1 Meq. per day in the stools.

Both patients behaved in a similar manner in regard to their metabolism of sodium and chloride. In all four foreperiods (dotted lines in the charts), when diets were not analyzed, there was exhibited considerable deficit of these two elements. It was assumed that the patients had, in each instance, been on a higher salt intake previously, and that the deficit was but the salt loss commonly seen in normal persons when they are shifted to diets containing less sodium chloride than they had previously ingested.

The period of abrupt starvation resulted, in both patients, in sharp sodium losses (75 and 65 Meq. per day, respectively), with losses of chloride of 55 and 65 Meq. Further loss of these elements ceased in the next succeeding period on the small intake of 25 Meq. (approximately 2 gms. NaCl per day), and repletion of about one-half the loss due to the starvation days was achieved in the four subsequent days with increasing intake, a sort of "rebound phenomenon." With further dietary increments (above normographic), P.T. merely remained in sodium and chloride equilibrium and did not continue to store these elements. H.T. even reverted to a small NaCl deficit during the two days that he received one and one-half times the initial diet complement.

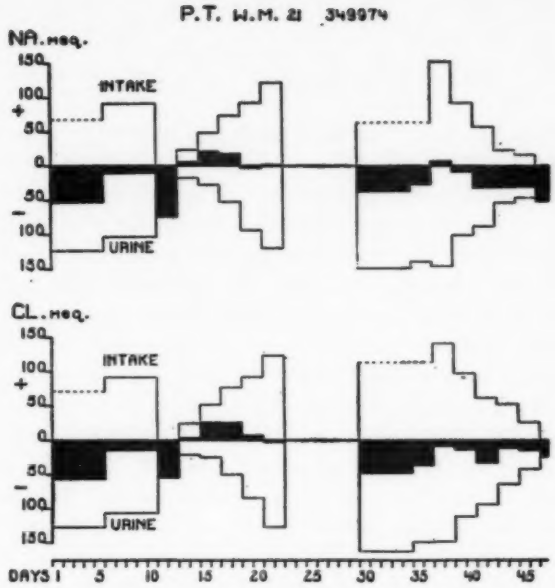


CHART VIII

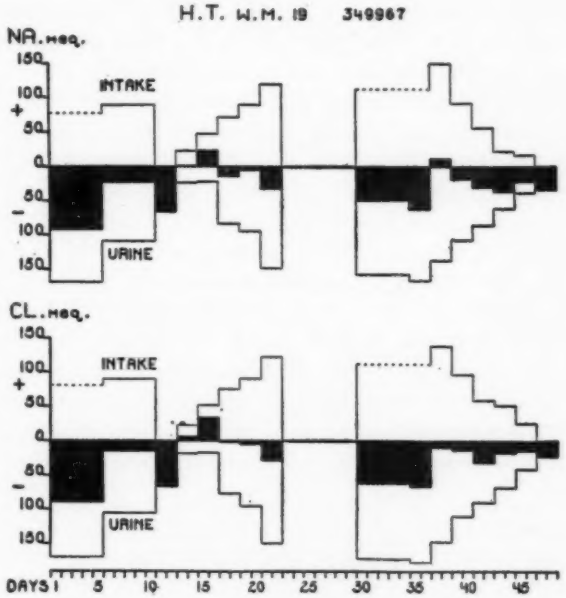


CHART IX

CHARTS VIII AND IX

NaCl balance.

During the periods of diminishing dietary intake to starvation, both patients lost sodium and chloride; but here the movement of the two elements seemed to diverge slightly. In both cases more sodium than chloride was lost; the sodium losses for the total period on P.T. being 294 Meq., on H.T. 270 Meq., whereas the chloride losses were 202 and 217 Meq., respectively. The ratios here represented between sodium and chloride approximate those occupied by these elements in the extracellular body fluids.

Alterations in blood constituents. As the result of starvation, both after the abrupt 2-day period (days 11–12) and at the end of the gradual depletion (day 48), there was evidence of minimal concentration of the solid elements of the blood, as reflected in RBC count, WBC count, hematocrit or total plasma protein concentration. Except for the plasma protein changes, the alterations are scarcely greater than the error of the methods used. Plasma protein concentration, as a result of the abrupt 2-day fast, fell from 7.65 to 6.53 gms. per 100 cc., in the case of H.T., whereas it rose from 7.22 to 7.40 in P.T. During the more prolonged graduated starvation, the plasma protein concentration rose in both cases; in H.T. from 7.6 to 8.15, in P.T. from 6.92 to 7.43. Albumen and globulin fractions were not determined.

Non-protein nitrogen rose slightly in both patients after both the brief fast and the more prolonged graduated fast.[5] Despite the rise in N.P.N., the urea nitrogen fell slightly or remained stable. There was a sharp rise in plasma uric acid resulting from both types of reduction of intake in both patients; during the 2-day fast it rose from 5.3 mgm. to 8.0 mgm. per 100 cc. plasma and from 5.3 to 7.2 mgm. During the slower longer undernutrition period (5/31 to 6/14), the elevation of uric acid was even greater, from 5.5 to 10 mgm. in P.T. and from 5.1 to 8.0 mgm. in H.T.

Sharp reductions in the concentrations of serum bicarbonate and cloride resulted from both types of fasting. In the two-day fast, serum CO_2 fell from 29.2 to 17.9 Meq. per litre in one patient and from 30.4 to 24.2 in the other. During the more graduated longer period

[5] Since urea is freely diffusible in body water, a rise in concentration of N.P.N. must be accounted for by breakdown of protein and in individuals of average size, 0.5 gms. protein nitrogen broken down will, if not excreted, cause a rise of 1 mgm. N.P.N. Fluctuations in N.P.N. during these experiments were small and have not been calculated in the nitrogen balances.

of undernutrition to the fasting point, the serum CO_2 fell from 29.2 to 21.6 Meq. per litre and from 29.2 to 25.8, respectively. Concentration in the serum of calcium, phosphorus and potassium changed but little throughout the experients. Serum sodium concentration fell in P.T. from 140 to 134 Meq. per litre after the two days' fast and from 135 to 132 in the period of more graduated reduced feeding. In the case of H.T., the fall in sodium concentration was minimal.

TABLE 5

P.T.

DATE	DAY	SERUM										BSP		BLOOD			
		T.P.	NPN	Urea	CO_2	Cl	Ca	P	Na	K	Uric acid	5 min.	30 min.	RBC	Hgb.	Hct.	WBC
5/2	5	7.22	27	15	29.2	102.0	9.80	3.48	140.0	4.1	5.3	52	3	5.08	16.0	47.0	5600
5/9	12	7.40	33	12	17.9	98.5	9.60	3.92	134.0	3.6	8.0	75		5.41	16.3	49.5	7000
5/21	25	7.40	29	18	29.2	105.0	9.60	4.20	140.0	4.1		69	4	5.23	15.3	45.8	4200
5/31	35	6.92	32	15	29.2	102.0	10.10	4.31	135.0	3.7	5.5	46	4	5.52	15.5	44.2	4275
6/14	49	7.43	35	12	21.6	102.0	9.46	3.64	132.3	3.4	10.0	77	15	5.26	15.7	46.0	5650

TABLE 6

H. T.

DATE	DAY	SERUM										BSP		BLOOD			
		T.P.	NPN	Urea	CO_2	Cl	Ca	P	Na	K	Uric acid	5 min.	30 min.	RBC	Hgb.	Hct.	WBC
5/2	5	7.65	27	17	30.4	102.0	10.0	3.81	137.2	3.9	5.3	58	3	5.11	15.5	47.2	7900
5/9	12	6.53	38	17	24.2	96.4	10.1	4.91	136.0	4.0	7.2	74	10	5.66	16.0	50.2	7250
5/21	25	7.28	37	21	30.0	104.0	9.7	3.96	137.7	3.9		65	3	5.57	15.5	48.6	5250
5/31	35	7.60	32	20	29.2	98.6	11.2	4.71	136.3	3.6	5.1	79	7	5.16	15.0	45.5	6900
6/14	49	8.15	29	17	25.8	97.0	11.2	4.00	134.0	3.9	8.0	87	12	5.19	16.0	47.3	6100

Tables 5 and 6 give the results of the examinations made on the blood morphology and the blood constituents reported above.

Tests were made at periodic intervals during the experiments on the efficiency with which liver removed bromsulphthalein from the blood, 5 mgm. of the dye having been injected per kilogram of body weight; and observations taken at 5 and 30 minutes thereafter. After each period of fasting, reduction of liver function, as reflected by this test, was noted (Tables 5 and 6).

TABLE 7

	NITROGEN					POTASSIUM					CALCIUM					PHOSPHORUS					SODIUM					CHLORIDE		
Days	Diet	Urine	Stool	Total	Bal.	Diet	Urine	Stool	Total	Bal.	Diet	Urine	Stool	Total	Bal.	Diet	Urine	Stool	Total	Bal.	Diet	Urine	Stool	Total	Bal.	Diet	Urine	Bal.
1–5	10.00	13.95	.397	14.347	−4.35	80.47	68.5	3.24	71.14	+8.7	876	163	348	511	+365	1163	1151	190	1341	−178	68.5	121.8	.69	122.49	−53.9	70.6	126.8	−56.2
6–10	10.54	11.67	.932	12.60	−2.06	69.04	63.6	9.4	73.0	−4.0	970	137	932	1069	−99	1221	883	453	1336	−115	91.4	103.2	1.90	105.1	−13.7	91.9	106.2	−14.3
11–12	0	11.72	.26	11.98	−11.98	0	31.6	1.50	33.10	−33.1	0	140	266	406	−406	0	879	146	1020	−1020	0	74.7	.13	74.83	−74.8	0	54.8	−54.8
13–14	2.71	11.22	.26	11.48	−8.77	17.49	25.5	1.5	27.0	−9.5	232	171	266	437	−205	304	751	141	892	−588	24.1	17.4	.13	17.53	+6.5	24.7	20.5	+4.2
15–16	5.67	9.55	.48	10.03	−4.36	38.5	39.4	4.5	43.90	−5.4	475	151	499	650	−175	630	671	307	978	−348	49.6	27.3	.36	27.66	+21.9	51.8	25.0	+26.8
17–18	8.44	9.25	2.00	11.25	−2.81	49.1	43.8	19.5	63.3	−14.2	776	132	2140	2272	−1496	738	620	1151	1771	−1033	73.0	51.0	3.40	54.4	+18.6	76.6	49.8	+26.8
19–20	10.54	9.79	.41	10.20	+0.34	69.0	53.6	4.1	57.7	+11.3	970	152	409	561	+409	1221	783	211	994	+227	91.4	94.3	.62	94.92	−3.5	91.9	84.4	+7.5
21–22	12.86	11.89	.37	12.26	+0.60	90.7	70.3	3.4	73.7	+17.0	1244	191	371	562	+682	1598	926	200	1126	+472	121.7	119.6	1.02	120.6	+1.1	123.6	126.0	−2.4
23–24	15.93	12.42	1.02	13.44	+2.47	111.1	80.4	8.5	88.9	+22.2	1398	173	1008	1180	+217	1807	942	580	1522	+285	—	129.5	.79	130.3	—	—	—	—
30–34	15.03	13.30	.91	14.21	+.82	111.3	90.5	7.8	98.3	+13.0	1382	222	1065	1287	+95	1705	1003	613	1616	+89	113.2	149.6	.66	150.3	−37.1	113.7	161.9	−48.2
35–36	15.03	12.23	1.17	13.40	+1.63	111.3	87.4	7.8	95.2	+16.1	1382	204	1521	1725	−343	1705	958	843	1801	−96	113.2	139.3	.63	139.9	−26.7	113.7	148.8	−35.1
37–38	15.45	12.73	1.15	13.88	+1.57	95.4	79.7	8.6	88.3	+7.1	1360	228	1503	1731	−371	1731	880	861	1741	−10	152.4	145.2	.62	145.8	+6.6	140.7	148.4	−7.7
39–40	11.15	11.70	1.45	13.15	−2.00	72.6	68.4	12.3	80.7	−8.1	1179	209	1541	1750	−571	1200	946	880	1826	−626	92.8	100.4	.86	101.3	−8.5	98.0	110.3	−12.3
41–42	8.86	10.39	.72	11.11	−2.25	53.8	56.1	9.7	65.8	−12.0	791	194	661	855	−64	941	826	349	1175	−234	56.7	87.4	.97	88.4	−31.7	60.7	92.5	−31.8
43–44	5.88	9.98	.50	10.48	−4.60	35.9	42.4	6.4	49.3	−13.4	548	180	296	476	+72	620	765	165	930	−310	23.0	53.9	.37	54.3	−31.3	51.5	62.0	−10.5
45–46	2.74	9.09	.37	9.46	−6.72	16.1	22.1	3.8	25.9	−9.8	253	181	201	382	−129	297	703	121	824	−527	15.9	46.4	.37	46.8	−30.9	25.8	40.3	−14.5
47–48	0	9.37	—	9.37	−9.37	0	22.3	—	22.3	−22.3	0	212	—	212	−212	0	750	—	750	−750	0	51.4	—	51.4	−51.4	0	24.5	−24.5

TABLE 8

DAYS	NITROGEN					POTASSIUM					CALCIUM					PHOSPHORUS					SODIUM					CHLORIDE		
	Diet	Urine	Stool	Total	Bal.	Diet	Urine	Stool	Total	Bal.	Diet	Urine	Stool	Total	Bal.	Diet	Urine	Stool	Total	Bal.	Diet	Urine	Stool	Total	Bal.	Diet	Urine	Bal.
1–5	9.83	12.76	.66	13.42	−3.58	79.1	67.4	5.6	73.0	+6.1	862	210	679	889	−27	1144	949	407	1356	−212	78.6	169.3	1.3	170.6	−92.0	80.6	170.5	−89.9
6–10	10.36	11.96	.77	12.73	−2.37	67.9	65.2	6.2	71.4	−3.5	954	176	882	1058	−104	1201	1020	511	1531	−330	89.9	108.6	3.4	112.0	−22.1	90.3	105.1	−14.8
11–12	0	11.62	.20	11.82	−11.82	0	43.2	2.3	45.5	−45.5	0	165	148	313	−313	0	844	103	947	−947	0	65.6	.2	65.8	−65.8	0	65.5	−65.5
13–14	2.66	13.24	.20	13.44	−10.78	17.2	35.6	2.3	37.9	−20.7	228	208	148	356	−128	299	882	103	985	−686	23.7	22.5	.2	22.7	+1.0	24.3	18.0	+6.3
15–16	5.58	8.63	.75	19.38	−3.80	37.9	34.5	10.3	44.8	−6.9	468	158	781	939	−471	619	712	533	1245	−626	48.8	22.0	2.4	24.4	+24.4	51.0	16.5	+34.5
17–18	8.29	10.40	2.89	11.29	−3.00	48.2	47.6	7.9	55.5	−7.3	763	186	1094	1280	−517	726	716	671	1387	−661	71.8	83.6	2.5	86.1	−14.3	75.3	76.5	−1.3
19–20	10.36	11.16	—	11.16	−0.80	67.9	60.3	0	60.3	+7.6	954	207	0	257	+747	1200	809	—	809	+391	89.9	94.9	—	94.9	−5.0	90.3	95.2	−4.9
21–22	16.64	14.91	.98	15.89	−3.25	89.2	80.0	13.0	93.0	−3.8	1223	265	1477	1742	−519	1571	1032	954	1986	−415	119.7	148.7	2.5	151.2	−31.5	121.6	150.1	−28.5
23–24	15.66	15.59	1.77	16.36	−0.70	109.2	88.3	7.3	95.6	+13.6	1374	267	709	976	+398	1777	1028	452	1480	+297	—	139.7	1.8	141.5	—	—	149.6	—
30–34	14.77	14.41	.88	15.29	−0.52	109.4	90.5	9.5	100.0	+9.4	1359	319	1365	1684	−325	1676	989	868	1857	−181	111.3	157.3	3.2	160.5	−49.2	111.8	173.2	−61.4
35–36	14.77	15.73	1.16	16.89	−2.12	109.4	87.5	10.1	97.6	+11.8	1359	315	1477	1792	−433	1676	1110	963	2073	−397	111.3	169.1	6.7	173.8	−62.5	111.8	178.1	−66.3
37–38	15.19	13.63	.67	14.30	+0.89	93.8	80.7	6.7	87.4	+6.4	1337	313	827	1140	+197	1702	1024	496	1520	+182	149.8	136.8	2.3	139.1	+10.7	138.4	148.2	−9.8
39–40	10.96	14.89	.67	15.56	−4.60	71.3	72.4	6.7	79.1	−7.6	1159	329	827	1156	+3	1180	1132	496	1628	−448	91.3	108.0	2.3	110.3	−19.0	96.3	110.3	−14.0
41–42	8.71	12.39	.63	13.02	−4.31	52.9	59.0	6.8	65.8	−12.9	778	271	587	858	−80	926	827	354	1181	−255	55.7	85.5	.5	86.0	−30.3	59.7	89.9	−30.2
43–44	5.78	10.90	.65	11.55	−5.77	35.3	40.9	10.0	50.9	−15.5	539	270	570	840	−301	610	756	435	1191	−581	22.9	60.6	.6	61.2	−38.3	50.7	68.4	−17.7
45–46	2.69	10.95	—	10.95	−8.26	15.8	34.7	0	34.7	−18.9	249	248	—	248	+1	292	769	—	769	−477	15.6	38.9	—	38.9	−23.3	25.3	40.3	−15.0
47–48	0	11.98	—	11.98	−11.98	0	25.8	0	25.8	−25.8	0	278	—	278	−278	0	946	—	946	−946	0	34.7	—	34.7	−34.7	21.9	21.9	−21.9

DISCUSSION

These studies on nitrogen metabolism during regimes of undernutrition yield corroboration for the hypothetical concepts previously expressed (8). Graphic representation of the total nitrogen balance of these two healthy young men at bed rest, corrected by a factor derived from their body surface area, closely approximates the predicted nitrogen wastage. It had been observed previously, during preoperative control periods (25), that healthy and vigorous persons when put to bed and fed diets containing 10 grams nitrogen and 1600 calories per 1.73 sq. meters of surface area, would lose on an average of 2 to 3 grams nitrogen per day. The present studies show that, during brief periods of starvation and graduated increasing dietary, patients of this type lose but slightly more nitrogen per day, at *each* undernutrition level, than represented by the hypothetical standard "normographic" line. It has also been noted (5, 25) that patients debilitated either by chronic illness or undernutrition, were in positive nitrogen balance of 2 to 3 grams per day under identical conditions of bed rest and diet of 10 grams nitrogen and 1600 calories per 1.73 sq. m. Whether such depleted individuals, when subjected to starvation, would lose less nitrogen than normographic prediction is not known.

Accurate knowledge of the amount of nitrogen a patient will excrete at bed rest on a given dietary is of considerable practical interest. Such knowledge will permit evaluation of the *extra* nitrogen lost as the result of his disease or injury, i.e., from the so-called "protein catabolism reaction" or the "toxic destruction of protein."

The amount of protein wasted by the protein catabolism reaction must be a reflection of the intensity of the stimulus and the capacity of the individual to respond to the stimulus (2). Both these factors are exceedingly difficult to define and measure. As to the former, Peters and his coworkers (6B) have shown that, in some instances of acute infections, the increased protein breakdown continues long after such clinical manifestations as fever and leucocytosis have subsided. We have observed similar, though briefer, refractory periods after malarial chills, when, for 24 hours or more after clinical evidences of the paroxysm have past, the organism cannot normally accept and utilize for storage nitrogenous pabulum, either ingested or received parenterally; but excretes it almost quantitatively in the urine (7). We know that, other conditions being equal, a fracture results in much

greater wastage of nitrogen than does an osteotomy (1); and in like manner operations to correct inguinal hernias were associated with much less nitrogen loss than were appendectomies (6B).

From some preliminary observations, the capacity to respond to the stimulus seems closely linked with the nitrogen metabolism at bed rest. Those patients who tend to store nitrogen easily do not respond to trauma with much wastage of nitrogen; those who tend to waste nitrogen at bed rest are the ones who respond to trauma with seemingly lavish nitrogen expenditure (29).

Other features of the observations on these healthy young men during starvation deserve consideration. It would seem that with undernutrition dietary at fixed or falling levels, nitrogen and potassium are lost in the same proportions as they exist in muscle protoplasm. During periods of rising dietary after starvation, there is a tendency to hold and restore potassium earlier than nitrogen. Phosphorus and nitrogen move together in proportions closely approximating their concentrations in muscle protoplasm.

SUMMARY

Two healthy young male volunteers were observed during periods of (A) acute starvation and graduated increased feedings, and (B) graduated reduced feedings to a brief period of starvation. The pattern of metabolism of nitrogen, potassium, phosphorus, sodium and chloride were followed, together with observations on weight, concentrations of chloride, CO_2, sodium, potassium, non-protein nitrogen, uric acid and protein in the serum, blood morphology and bromsulphthalein liver function test. Large deviations from this pattern, if found in acutely traumatized, healthy individuals, may be assumed to result from the process of injury. Nitrogen losses, both at bed rest and during starvation, were found to be slightly greater than would be expected from a previously reported hypothetical chart. During periods of decreasing dietary, the nitrogen, potassium and phosphorus were lost in the same proportions as they have been found to exist in muscle protoplasm. During periods of increasing dietary, potassium was held earlier and in greater proportion than was nitrogen; phosphorus and nitrogen balances, however, continued to reflect their relative proportions in normal muscle.

Urinary excretion of alpha amino nitrogen tended to fall when the

patients were on submaintenance diets, below one-half the calculated requirements.

During periods of starvation, there were conspicuous losses of sodium and chloride, and the repletion of these substances was slow even when greater than maintenance diets were given.

Sharp reductions in concentrations of bicarbonate and chloride in the serum were exhibited as the result of both abrupt fasting and slow reduction in the diet.

Fasting reduced the capacity of the liver to remove bromsulphthalein from the blood.

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STUDIES ON THE PHARMACOLOGY OF DDT (2,2 BIS-PARACHLOROPHENYL-1,1,1,TRICHLOROETHANE)

The Chronic Toxicity of DDT in the Dog

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The acute toxicity of DDT has been discussed in a preceding communication from this laboratory (1). Several reports have been published dealing with the symptoms of chronic DDT intoxication. Smith and his co-workers from the U. S. Public Health Service noticed a moderate degree of liver damage taking the form of a centrolobular necrosis (2). Calvery and associates in studying the subacute toxicity of DDT in rats, mice, rabbits and guinea pigs described a hyperirritable stage followed by depression and death (3). The present study deals with an analysis of the effect of prolonged administration of DDT on the renal, and hepatic function of unanaesthetized dogs as well as a description of the effect of this drug on the central nervous system.

EXPERIMENTAL PROCEDURES

The experiments were performed on a total of 28 unanaesthetized dogs. A 10% solution of DDT in peanut oil was given by stomach tube. From 100 to 450 mg. of the drug were administered, 6 days a week, unless the condition of the animal was such that an additional dose would have jeopardized its life. Neurological symptoms were studied in all of these dogs. Renal function was observed in 3, hepatic function in 12 dogs. The period of observation varies from 2 weeks to 7 months.

The effective renal plasma flow was determined by means of the clearance of p-aminohippuric acid (4). Creatinine clearance was used to measure glomerular filtration (5). The maximal tubular excretory mass was determined as the p-aminohippuric acid TM.

The following methods were used to investigate hepatic function:

a. The quantitative determination of serum bilirubin by the method of Malloy and Evelyn (6).

b. A newly developed modification of the bromsulfalein test. This

colorimetric method, which will be described in detail in a later communication, was based on the finding that plasma precipitation with half saturated ammonium sulfate effectively reduced the turbidity of the plasma sample without interfering with quantitative recoveries of bromsulfalein. In performing this test, a single blood sample was drawn 10 minutes after intravenous injection of 15 mg. of the dye/kg. body weight and the plasma concentration of the dye was determined by means of a photo-electric colorimeter.

c. The formaldehyde gel test. A positive outcome of this test indicates alterations in the serum globulin fraction of the blood and has been routinely used to diagnose hepatic damage (7). The cephalin flocculation test (8) was tried and abandoned because of the high rate of positive reaction in normal dogs.

EXPERIMENTAL RESULTS

Neurological symptoms produced by DDT.

Twenty-eight dogs were studied over a period varying from 2 weeks to 5 months. Daily doses of 100 mg/kg. initiated moderate coarse tremor, commencing about 4 to 6 hours after ingestion of DDT. This disappeared, however, upon withdrawal of the drug. The daily administration of from 150 to 250 mg./kg. of DDT, on the other hand, resulted in the production of more severe neurological disturbances. The tremors were intense and seemed to involve all muscle groups. There was slight exaggeration of the stretch reflexes. Most pronounced were the aberrations in gait. The animals walked stiffly with extended hind and forelegs and overstepping was frequently noticed. The placing reactions were exaggerated. It seems significant that after several weeks of administration, withdrawal of the drug resulted in cessation of the tremors while the other neurological symptoms described above persisted for one or two days. Most of the animals, however, recovered fully after the drug had been discontinued.

When DDT was given in doses exceeding 250 mg./kg., persistent neurological signs developed sometimes after less than 3 weeks of administration. Five to six days after the last dose of DDT had been administered the animals still showed static and kinetic tremor. They characteristically exhibited severe hypermetria, lifting their feet abnormally high at each step. The muscular tone was increased and all

extremities exhibited a strong positive supporting reaction. On standing, the legs were rigid, hyper-extended and abnormally abducted. On backward extension of the head of the animals the tone of the forelimbs was greatly increased. Tilting the head to the right resulted in extreme extension of the right leg, tilting the head to the left in extreme extension of the left extremities. Similar exaggerations of the attitudinal reflexes have been observed by Rademaker in decerebellate animals (9). Of special interest were the aberrations in gait. The dogs were unable to walk straight but tended instead to proceed in a zigzag fashion. Frequently a striking inability to arrest forward locomotion was evident, with the result that the animals crashed into clearly visible objects. After a fall, the dogs had difficulty in getting on their feet, frequently falling repeatedly before achieving their aim. At this stage of irreversibility the tolerance to DDT decreased markedly. Forty mg./kg. of the drug which ordinarily had only slight or no effect produced most severe symptoms in these animals. The dogs were unable to walk, and lay prostrate on the floor. Coarse tremor, exextensor rigidity and hyperextension of the neck muscles were present. Their inability to eat resulted in severe weight loss and dehydration. Occasionally animals developed severe corneal lesions which were probably caused by their inability to close their eyelids. It was apparent from these observations that irreversible symptoms had been produced by the prolonged administration of DDT. Their clinical nature indicated that injury to the cerebellum might have played a major rôle in their production.

EXPERIMENTS ON RENAL AND HEPATIC FUNCTION

The glomerular filtration rate and effective renal plasma flow and maximal tubular excretory mass were studied in a series of 3 animals over a period ranging from 1 to 3 months (tables I to III). Although all the dogs were followed throughout the development of chronic DDT poisoning until persistent neurological symptoms had developed, no deviation from normal control values was noticeable, indicating that prolonged administration of DDT had failed to produce renal damage (tables I to III).

On the other hand the results of the quantitative bilirubin and the formaldehyde gel tests reveal some degree of hepatic damage (table

IV). A total of 15 dogs was used for liver function tests. These animals had received DDT over a period of from 2 weeks to 5 months. The formaldehyde gel test showed a positive reaction in 7 out of 10

TABLE I

The effect of oral ingestion of DDT on renal function

Dog #7

DATE	GLOMERULAR FILTRATION RATE	EFFECTIVE RENAL PLASMA FLOW	PNH_2TM	FILTRATION FRACTION
	cc./min.	*cc./min.*	*mg./min.*	
30 Nov. 1944	61.8	143	14.95	0.43
2 Dec. 1944	66.2	144	20.8	0.46
11 Jan. 1945	DDT started			
23 Jan. 1945	55.98	159.16	15.78	0.35
13 Feb. 1945	44.6	136.5	19.4	0.33
23 Feb. 1945	46.5	150.3	19.92	0.29
12 Mar. 1945	65.0	192	19.0	0.34

PNH_2TM = para-amino hippuric acid TM.

TABLE II

The effect of oral ingestion of DDT on renal function

Dog #4

DATE	GLOMERULAR FILTRATION RATE	EFFECTIVE RENAL PLASMA FLOW	PNH_2TM	FILTRATION FRACTION
	cc./min.	*cc./min.*	*mg./min.*	
7 Dec. 1944	53.2	123.5	21.8	0.43
15 Dec. 1944	38.8	163	10.5	0.24
22 Dec. 1944	43.79		7.65	0.28
11 Jan. 1945	DDT started			
24 Jan. 1945	54.5	167	12.8	0.33
14 Feb. 1945	58.5	162	12.8	0.36
22 Feb. 1945	45.4	210.6	32.5	0.21
9 Mar. 1945	53.0	176.6	24.97	0.30

PNH_2TM = para-amino hippuric acid TM.

animals (table IV). Usually the test became positive in from 2 to 3 weeks after ingestion of the drug. In one animal (#6) the test was positive during the control period (table IV). As a positive formaldehyde gel test is commensurate with a disturbance in the globulin

fraction of the plasma, the assumption might be ventured that the positive test obtained in these experiments is indicative of some degree of liver damage. Further evidence of liver damage was obtained in 4 animals, which showed elevated serum bilirubin levels of from 0.7 to 1.6 mg./100 cc. of serum, shortly before the death of the animals. It is probable that the failure to detect bilirubinemia in the rest of the animals was caused by the fact that the dogs were sacrificed before terminal hepatic damage had developed.

Results obtained with the quantitative bromsulfalein test were inconsistent. When the values obtained during the development of DDT intoxication were compared with bromsulfalein retention in a

TABLE III

The effect of oral ingestion of DDT on renal function

Dog #8

DATE	GLOMERULAR FILTRATION RATE	EFFECTIVE RENAL PLASMA FLOW	PNH_2TM	FILTRATION FRACTION
	cc./min.	*cc./min.*	*mg./min.*	
26 Nov. 1944	85.1	159.33	26.65	0.53
29 Nov. 1944	73.7	231.5	14.8	0.33
11 Jan. 1945	DDT started			
25 Jan. 1945	71.66	183	26.5	0.39
5 Feb. 1945	77.7	270.33	27.1	0.29
11 Feb. 1945	72	252	20.3	0.28

PNH_2TM = para-amino hippuric acid TM.

series of 23 normal animals, it was found that significant dye retention did not occur at any time. A slight degree of early impairment of liver function, however, may exist in the early stages of chronic DDT intoxication, as shown by an early positive formaldehyde gel test.

DISCUSSION

The studies reported in this paper demonstrate that the oral ingestion of relatively small amounts of DDT in oil (100 mg./kg. weight) produces only mild tremor in unanaesthetized dogs. In larger doses (150 to 250 mg./kg.) severe neurological symptoms can be observed. The animals develop severe static and kinetic tremors of all muscle groups and the stretch reflexes are exaggerated. On walking, the dogs

TABLE IV

Results of liver function tests

DOG NO.	DDT ADMIN. BEGUN	FEB. 5	FEB. 14	MAR. 1	MAR. 14	APR. 6		APR. 11		APR. 14	APR. 16			APR. 17	APR. 26		MAY 4			MAY 10		JULY 22
		Bil.	Bil.	Bil.	Bil.	BSP	F.G.	Bil.	F.G.	BSP	BSP	F.G.	Bil.	BSP	BSP	F.G.	BSP	F.G.	Bil.	BSP	F.G.	BSP
	1945	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*mg. %*		*mg. %*		*mg. %*	*mg. %*		*mg. %*	*mg. %*	*mg. %*		*mg. %*		*mg. %*	*mg. %*		*mg. %*
1	Apr. 11									0.32					0.14	3+				0.20	3+	
2	Apr. 11										0.25	4+	−	0.37	0.18	3+				0.18	3+	0.68
3	Apr. 11								−		0.36	−	−		0.20	−					3+	
4	Jan. 11		1.0	0.8	1.6																	
5	Apr. 16																			0.50		
6	Apr. 16																0.46	4+	−			
7	Jan. 11			0.7	0.7																	
8	Jan. 11	1.4																				
9	Mar. 20						1+															
10	Apr. 7								±													
11	Apr. 11										0.18	4+	−	0.18	0.14	3+						
12	Mar. 14					0.52	2+															
13	Mar. 14					0.32	−	0.7	−													
14	Mar. 20					0.46	−		−		0.64	−	−		0.29	−					−	
15	Mar. 21					0.57	−		−		0.36	4+	−		0.42	3+				0.38	3+	

Bil.—Bilirubin.

F.G.—Formaldehyde gel reaction.

BSP—Bromsulfalein test.

Notes:

(1) The serum bilirubin level in normal dogs does not exceed 0.5 mg per cent.

(2) The plasma bromsulfalein retention in 23 normal dogs was 0.356 mg. per cent with a normal equivalent deviation of 0.1 mg. per cent.

show overstepping and hyperextension of the limbs. When the placing reactions of the dogs are tested by holding them in the air with their vision excluded, the slightest contact of the margin of a table with the anterior aspect of their feet results in greatly exaggerated flexion of the limb, followed by maximal extension. When the administration of DDT is discontinued after several weeks, the aberrations in gait and the positive placing reaction persist for one or more days.

The oral ingestion of DDT in doses exceeding 250 mg./kg. for a period of from 2 to 4 weeks produces more persistent neurological disturbances. Some of the dogs continue to exhibit severe tremors and hypermetria. On standing, their legs are rigid and hyperextended. In addition the attitudinal reflexes, especially the tonic neck reflexes are increased in intensity. The aberrations in gait are equally pronounced. The dogs are unable to maintain a constant direction, falling repeatedly from one side to the other before they are able to right themselves. During this pronounced state of DDT intoxication small amounts of the drug (40 mg./kg.) lead to extremely severe central nervous symptoms with hyperextension of the neck muscles.

It is evident from this description that ataxia is the most outstanding neurological manifestation of chronic DDT intoxication. This ataxia is similar to that described by Dusser de Barenne (10) and by Fulton (11) in the decerebellate animal. According to these investigations a positive supporting reaction as well as an overactivity of the attitudinal reflexes represent characteristic manifestations of decerebellate dogs. It is, therefore, probable that one of the main actions of DDT in the dog is on the cerebellum. The production of irreversible neurological symptoms indicates the development of anatomical lesions in that organ, particularly the dentate nucleus (12).

The results of the renal function tests demonstrate that prolonged administration of DDT is without effect on either glomerular filtration rate or effective renal plasma flow (tables I to III). Hepatic function, however, is affected early by the drug since the formaldehyde gel test, which reflects changes in the plasma globulin fraction, (7) becomes positive in from 2 to 3 weeks after feeding of DDT is started (table IV). It can be assumed that this represents an indication of early hepatocellular damage. It is evident that the liver damage is not severe, at least in the early stages of chronic intoxication, as the quantitative

bromsulfalein test shows no retention of the dye. As some of the animals showed some increase in the serum bilirubin, the assumption might be ventured that severe liver damage is a terminal occurrence, although slight impairment of hepatic function can exist in the early state of chronic DDT intoxication.

SUMMARY

1. DDT in large doses produced irreversible neurological disturbances. The nature of these symptoms indicated that the drug acted primarily on the cerebellum.

2. DDT produced a moderate degree of liver damage, which in some instances became terminally more severe. DDT had no effect on renal function.

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PROCEEDINGS OF THE MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

Held in the Hurd Memorial Hall, May 14, 1945

Experiences in the Psychotherapy of Obsessive-Schizophrenic Personalities. Barbara J. Betz (Department of Psychiatry).

The problem of how the therapist can effectively treat the patient whose personality reaction to others is dominated by the schizophrenic pattern of emotional withdrawal and only guarded particpation in any personal relationship is considered. The strategic goal of therapy lies in the healthy emotional growth of the patient in the direction of an increased desire for and capacity to participate with self-assurance in emotional relationships with others. Therapeutic tactics used in treating a series of eight patients whose emotional adjustments to others improved sufficiently to seem to warrant the termination of further active treatment are discussed.

The characteristic personality pattern of each of these eight patients in relationships with other people was schizophrenic but some contact with others was maintained by the operation of an obsessive pattern, as evinced, for instance, by perfectionistic discharge of duty. Seven cases were out-patients seen in one hour therapeutic conferences once a week. One hospitalized case was seen in one hour conferences twice a week. The average total number of conferences was 29. The time elapsed since treatment was terminated ranges from 6 months to three and one half years.

In each of these eight patients the course of treatment seemed to fall into three phases, each phase similar in all of the cases. *Phase one* might be called the period of *timid rigidity or restricted spontaneity*. The patient reacted in the relationship with the therapist by a markedly restrictive pattern, suppressing almost any sort of spontaneity and seeming to fear any inner feelings as threats to his personal security if incompatible with his particular habitual conception of himself and his significance to others. The patients' initial therapeutic objective was to strengthen the effectiveness of his habitual patterns rather than to increase his range of emotional expression. The therapist found the initial role in which the patient could tolerate a relationship to him sharply splinted by this timid rigidity. If, however, the therapist could become an integral part of the patients' emotional experience, in any way acceptable at all, the groundwork was laid for subsequent more satisfactory patterns of participation. The tactical approach permitting the development of such a significant preliminary relationship may be expressed by the phrase: *encouraging self-revealment*. If the therapist limited his early role essentially to that of interest and thoughtfulness about whatever attitudes or feelings the patient was ready to reveal, and so indicating a *desire to understand* rather than to make the

patient understand, a framework seemed to be provided for the formation of a relationship built up out of the actual fiber of the patients' existing personality patterns. These eight patients responded to this approach with the rapid development of a *feeling of being understood* and their insecurity in relation to the therapist diminished. As the patient became more secure his interest in the therapist's own reactions and attitudes toward him spontaneously increased. He became less timid and less rigid and the therapeutic relationship became redefined along more spontaneous lines.

Phase two of treatment might be called the period of *self-definition* or *spontaneity of mutual interaction*, the transition from the first phase occurring as the patient became more secure and spontaneous with the therapist, though not yet with others. The therapeutic role which proved particularly effective now may be expressed by the phrase: *encouraging self-evaluation*. Interest now was focused on what the patient himself really wanted for himself, quite apart from what others (including the therapist) might think advisable. The patient, confronted by this attitude of the therapist of consistent but not insistent confidence in his potential capacity for defining his own judgment. As this sharpening of his self-definition occurred, his security in relationships with people other than the therapist was enhanced, and the transition to the ending phase of treatment occurred.

Phase three might be called the period of *broadened social confidence and participation*. These patients began spontaneously to reject their earlier goal of maintaining withdrawal from others and to participate with increasing confidence in relationships with others. The therapist was now seen as representative of others rather than as special and more trustworthy. As this trend gained momentum, the need for a continuance of the therapeutic relationship automatically diminished. The therapeutic role during this phase was largely one of standing by, with continued interest in the patient's efforts to handle his own life, and appreciation both of the successes and difficulties he was encountering.

DISCUSSION

Dr. Jerome D. Hartz said that the results in these patients are very impressive. Perhaps the importance of the problem may be clearer if one realizes that one third of all of the hospital beds in the country are occupied by patients who have progressed from the stage which Dr. Betz describes.

Recent Studies on the Function of the Adrenal Cortex:

I. Biochemical Changes Associated with the Activity of the Adrenal Cortex.

C. N. H. Long (Yale University, School of Medicine, New Haven, Connecticut).

In spite of the large amount of work carried on in recent years the intimate mechanisms underlying adrenal cortical activity remain obscure.

Two important phases of the study of the function of any endocrine organ are: 1) the nature of the effect of its hormones upon biological processes, particularly

those of metabolism and 2) the conditions and circumstances underlying the formation and secretion of the characteristic chemical compounds that constitute its specific secretion or hormones.

Dr. White and I will touch upon both these problems as they relate to the adrenal cortex, but we should warn you that it is not yet possible to give complete answers to both these fundamental questions as they relate to this gland of internal secretion.

The determination of the conditions and circumstances under which the secretory activity of an endocrine gland may be altered can be approached both directly and indirectly.

The *direct* methods, while often presenting great practical difficulties, have the advantage that they record either the actual chemical changes in the gland associated with its activity or the level of its secretions in the body fluids, while the *indirect* methods, which usually involve weighing organs or following metabolic changes, suffer from the often present difficulty of distinguishing between the primary effect of the hormone and the secondary or compensating changes that may be invoked by its action. However, taken together or separately, both methods can be relied upon to yield valuable information.

Tonight, Dr. White and I propose to outline to you the results of studies of adrenal cortical function both by the direct and indirect methods. These have been carried out in collaboration with Dr. Sayers and Miss Fry, of our department, and with Dr. Dougherty, of the department of anatomy at Yale.

The degree of activity of an endocrine gland may be *directly* determined:

a) by alterations in the amount of hormone in the blood.

b) by alterations in the urinary excretion of the hormone or its known metabolites.

c) by alterations in content of the hormone in the gland

d) by alterations in the content of glandular constituents that are known to be associated with the formation and secretion of the hormone.

It may be *indirectly* determined:

e) by observation of the metabolic changes that are characteristic of the action of the hormone.

So far as the adrenal cortex is concerned, the following comments may be made:

a. *Level of hormone in the blood.* This has been followed by Vogt in the plasma of the adrenal vein of the dog. The hormone content was assayed by the method of Selye and Schenker.

b. *Urinary excretion.* The urinary metabolites of the cortical hormones are not well defined but probably appear as 17-keto steroids. Since these also arise in the metabolism of androgens, the method is not entirely as an indicator of cortical hormone secretion. Methods for the direct determination of urinary "cortin" have been worked out by Browne and Venning and by Dorfman and his colleagues. By these methods it has been shown that a variety of stresses increase the excretion of these hormones.

c. *Hormone in gland.* This is not a feasible procedure for the adrenal, due to the low content of hormone in the gland at any one time.

d. *Alterations in cholesterol and ascorbic acid.* So far as my contribution to this evening's discussion is concerned, I would like first to all to present the results of some experiments on the alterations in *adrenal cholesterol and ascorbic acid* that appear to be associated with its secretory activity. These substances are both present in high concentration in the adrenal, cholesterol to the extent of 4–5 g. %of the wet weight in the rat adrenal—nearly all in the ester form—while ascorbic acid is present to the extent of 4–500 mgs. % in this species and from 150–180 mgs. % in the guinea pig.

The fact that both adrenal cholesterol and ascorbic acid undergo wide variations under different circumstances has been reported and commented on by many authors, and numerous attempts to associate these changes with the alleged detoxifying action of the adrenal cortex have been made.

The key observation in the present series of experiments is that highly purified pituitary adrenotrophic hormone (A.C.T.), whose only known action in the body is the stimulation of the adrenal cortex, produces a marked fall in both adrenal cholesterol and ascorbic acid. Following the injection of quantities of this trophic hormone varying from 0.25–4.0 mgs. per 100 gms. of body weight, there occurs:

a) A rapid fall (60% in one hour) in ascorbic acid which in the rat returns to normal in about six hours. In the *guinea pig*, an animal that cannot synthesize ascorbic acid, while A.C.T. causes the same fall in adrenal ascorbic acid, the return to normal is much delayed unless ascorbic acid is given to the animal. This may be a point of importance when considering the consequences of severe adrenal cortical stimulation in *man* and perhaps bears on the unsolved question of the biochemical lesions in scurvy.

b) A delayed fall in the cholesterol level (50% in three to six hours) and in the rat a slower return to normal. In the guinea pig the cholesterol level is depressed for a longer period, although recovery is nearly complete in twenty-four hours.

That these changes are actually associated with a liberation of cortical hormones is shown by the observation that the liver glycogen rises during the period of the decline in adrenal ascobic acid and cholesterol, an effect characteristic of the corticosterones in the fasting rat. Similar changes in adrenal ascorbic acid and cholesterol and in liver glycogen are produced in rats three days after hypophysectomy. The adrenals of rats hypophysectomized for longer periods are sluggish in this type of response to A.C.T.

It should be noted that the adrenal of hypophysectomized rats is able to return its cholesterol content to normal after depletion by A.C.T. It also has a higher than normal content, so evidently A.C.T. is concerned either with the release of preformed cortical hormones or with their formation from cholesterol.

There are no significant changes in the cholesterol and ascorbic acid of other tissues such as brain, liver, muscle, and kidney when A.C.T. is injected.

Cholesterol and Ascorbic Acid as Procursors of Cortical Hormones. The propriety of the assumption that alterations in adrenal cholesterol and ascorbic acid are directly associated with the formation of the cortical hormones is considerably strengthened by two recent sets of observations:

a) It has been shown by Rittenberg and Bloch by use of cholesterol tagged with deuterium that this substance is not only converted into cholic acid but also into pregnandiol, the urinary metabolite of progesterone. Now progesterone has a very similar structure to the cortical steroids and strongly suggests that they have a common precursor—cholesterol.

b) A few years ago Zwemer, Lowenstein and Pines suggested that the cortical hormone, as secreted by the gland, was a complex composed of a steroid with a water soluble nucleus side chain which might be ascorbic acid. Lowenstein has recently reported to me the isolation from the adrenal of a water soluble biologically active steroid, in which ascorbic acid is apparently associated with a steroid nucleus. Confirmation of this interesting claim would do much to clarify the association of the changes in adrenal ascorbic acid with the activity of the gland.

The evidence is, therefore, suggestive that on stimulation, the adrenal cortex discharges its hormone as a compound associated with ascorbic acid and that cholesterol forms a reserve of steroid material that can rapidly be used to form further quantities of hormone. Such a hypothesis is in keeping with the observed effects of stimulation of the gland by A.C.T.

Adrenal Changes Following Endogenous Release of A.C.T. It is now generally recognized that the adaptation or compensation of the organism to a wide variety of stresses, whether applied from within or without, is followed by activation of the adrenal cortex and an increased secretion of its hormones. It should, however, be remembered with equal emphasis that such activation of the adrenal cortex *only occurs after an initial activation of the secretion of A.C.T. by the anterior pituitary.*

We have shown that the alterations in adrenal cholesterol and ascorbic acid in normal animals reflect their exposure to several types of stress and that this response is abolished by hypophysectomy. I would like to present three examples of this:

a) *Effect of Exposure to Cold.* Exposure of rats or guinea pigs to an environmental temperature of 1–2°C. for periods of one to four hours is followed by a rapid loss of ascorbic acid and a slower loss of cholesterol. This does not occur in hypophysectomized animals.

b) *Hemorrhage.* Rats, when subjected to a non-fatal hemorrhage equal to 2 % of body weight withdrawn in one hour, have lost 50% of adrenal ascorbic acid and 30 % of adrenal cholesterol at the end of the bleeding period. Further losses are sustained in the next few hours, and, although the ascorbic acid returns to normal as recovery proceeds, the cholesterol level is still 30% below normal some twenty-four hours later. No changes occur in the adrenals of hypophysectomized rats subjected to similar hemorrhages.

c) *Burns.* In conjunction with Dr. H. Harkins, we have studied the effect of hot water scalds on the adrenal cholesterol of rats. Such treatment is a violent stimulus to the adrenal cortex, as judged by the massive falls that occur in its cholesterol content. Similar scalds inflicted on hypophysectomized rats did not lower the adrenal cholesterol.

From the similarity of the response of the adrenal to these three stresses of dif-

ferent types, it can be assumed that all types of stress that activate the hypophyseal-adrenal mechanism bring about similar alterations in the adrenal cholesterol and ascorbic acid.

Mechanism of Activation of Anterior Pituitary—Adrenal Cortex in Response to Stress. Various types of stress evoke different types of response in the organism and require different metabolic adjustments that are reflected in the composition of the blood and body fluids. It is unlikely that the anterior pituitary is nonspecific in its response to changes in the composition of the blood traversing it, although the existence of some humoral change common to all types of stress cannot be denied.

We have, therefore, been attracted to the hypothesis that one common denominator of all types of stress that evoke an increased adrenal cortical secretion is the increased activity of the sympathetic nervous system with its concomitant discharge of epinephrine. So far we have been able to show that either the subcutaneous or intravenous injection of epinephrine in physiological amounts into normal rats is followed by a depletion of the adrenal cholesterol and ascorbic acid and that such injections are without effect on the adrenal of hypophysectomized animals. In a recent paper Vogt has also found that epinephrine increases the rate of liberation of cortical hormone from the adrenal of the dog.

The question, however, still remains to be answered as to whether this effect of epinephrine is due to a direct stimulation of the cells of the anterior pituitary that secrete A.C.T. or whether it is due to the effect of epinephrine on the conposition of the blood which in turn serves as the stimulus to pituitary secretion.

These experiments obviously raise the question as to the possible sympathetic innervation of the anterior lobe. It will probably be possible to use the alterations in adrenal cholesterol and ascorbic acid as indicators of anterior lobe activation after stimulation of various nerve pathways. This we are attempting to do at the present time. For the moment it can be said that neither adrenal demedullation, cervical sympathectomy, or a combination of both will prevent the fall in adrenal cholesterol and ascorbic acid after exposure to cold.

II. Pituitary-Adrenal Cortical Control of Normal and Immune Globulin Release from Lymphocytes. ABRAHAM WHITE (Yale University, School of Medicine, New Haven, Connecticut).

Data were presented which have been obtained in collaboration with Dr. T. F. Dougherty. The results permit the following conclusions:

1. The number of circulating lymphocytes is under pituitary-adrenal cortical control.

2. Pituitary-adrenal cortical secretion produces functional alterations in lymphoid tissue. The characteristic changes observed in lymphoid tissue following a single injection of either pituitary adrenotrophic hormone or a potent adrenal cortical extract are seen as early as 3 hours after injection and persist during a subsequent 18-hour period. These changes are acute ones, and normal lymphoid

tissue histology is generally restored within 24 hours after hormone administration. The histological alterations seen include:

a. Shrinkage and pyknosis of lymphocytes.

b. Accentuated budding of lymphocyte cytoplasm.

c. Dissolution of lymphocytes.

d. Accentuated development of macrophages.

3. Therefore, the lymphopenia seen following hormone administration is at least partially due to a failure of delivery of lymphocytes to the circulation.

4. The budding of the lymphocyte cytoplasm and the dissolution of lymphocytes results in a contribution of lymphocyte constituents to the lymph and thus to the serum. One of these constituents has been demonstrated to be identical with normal serum gamma globulin; a second constituent is probably identical with normal serum beta globulin. Thus, following a single injection of adrenotrophin or adrenal cortical steroids in rabbits, there occurs an increase in the total beta and gamma globulins of the serum. This increase prevails during the period of maximum lymphopenia and lymphocyte dissolution in the tissues.

5. In the immunized animal, lymphocytes contain labeled or immune globulin. The rate of release of this antibody to the circulation is likewise under pituitary-adrenal cortical control. One manifestation of this control is the anamnestic response, which is not observed in the absence of the adrenals.

6. These alterations in lymphoid tissue physiology occur after adrenotrophin injection or following injection of those adrenal cortical steroids oxygenated in position 11 of the steroid nucleus. Desoxycorticosterone acetate, in the doses used, has no effect on blood lymphocyte levels, lymphoid tissue histology, or serum globulin concentrations.

7. Many non-specific and unrelated stimuli, e.g., cold, anoxia, bacterial toxins, benzene, arsenic, have been shown to produce similar alterations in numbers of blood lymphocytes, lymphoid tissue histology, and release of normal and immune globulins. These aspects of lymphoid tissue physiology do not occur if the same stimuli are applied to the adrenalectomized or hypophysectomized animal.

8. The specific action of adrenal cortical steroids on lymphocytes as an end organ is seen from studies with x-rays. A dose of 10r will produce all of the above discussed changes in normal mice. None of these alterations occur in adrenalectomized animals. A dose of 200r, large enough to affect lymphocytes directly, will produce lymphopenia, lymphocyte dissolution, and serum globulin increases, even in the absence of the adrenals. Therefore, the physiological responses seen following the liberation of, or injection of, 11-oxygenated adrenal cortical steroids are a consequence of the specific action of these steroids on lymphocytes. Any agent which can affect lymphocytes directly, e.g., high doses of radiation, produces the same sequence of events.

9. The alterations and responses discussed are normal physiological processes whose rate is controlled by pituitary-adrenal cortical secretion. Acceleration of this rate, and consequent magnification of these alterations and responses, has been produced by injection of the hormones.

DISCUSSION OF DRS. LONG AND WHITE

Dr. Rich said that he had been interested for a long time in the lymphocyte and in its participation in the formation of antibody. The beautiful and exceedingly important work of Dr. Long and Dr. White is serving to clarify this fundamental question. It has long been believed that the macrophage, which so conspicuously takes up all sorts of foreign colloids, and therefore in all probability foreign antigens, also plays a part in antibody formation. Dr. Rich inquired whether Dr. White feels that the lymphocyte is the only participant in this reaction or whether he is still interested in the macrophage.

Dr. Harkins said that he particularly enjoyed the presentation of Dr. Long and Dr. White because of the opportunity a year and a half ago of working in their laboratory. At that particular time Dr. White's work on hormonal lymphopenia was just producing results. The vicarious thrill that goes through even the not-participating workers in a laboratory as occurred at that time can be especially appreciated by those of us in the surgery department here during the past few months. As an extension of the work of Dr. Long, Dr. Harkins has studied the fall in adrenal cholesterol and ascorbic acid following burns and tourniquet shock. Both items decrease markedly. This decrease does not occur in hypophysectomized animals, indicating a possible humoral mechanism. The effect of adrenaline injections also corresponded to humoral theories. To test this mechanism further, studies on adrenomedullectomized and adrenal transplanted rats gave equivocal results, possibly due to scar tissues. To complicate the picture further, in parabiotic animals only the traumatized partner showed a decrease in adrenal cholesterol, indicating as far as this goes a lack of humoral mechanism. Traumatic effects on the adrenal of a qualitative nature have been recognized for some time. The work of Dr. Long and Dr. White has put these changes on a firm chemical basis and they have given us several quantitative local and peripheral means of measuring such changes. This work should lead to still further elucidation of this important subject.

Dr. Wilkins said that he had been greatly impressed by the multiplicity of syndromes arising from pathological changes in the adrenals and apparently due to differences in the type of hormone elaborated by the adrenal in these various conditions. He asked whether Dr. Long would comment on the relation of the phenomena which he has discussed to patients with Cushing's disease and the adrenogenital syndrome.

Dr. Firor inquired whether the hormone which produced such rapid lymphoid dissolution had been tried in cases of lymphosarcoma and lymphatic leukemia.

Dr. White said in reply to Dr. Rich that he was definitely still interested in the macrophage.

It has been known for some time that fasting leads to lymphoid hyperplasia. We have noted that an increase in gamma globulin occurs as early as a second day of a fast and that the lymphocytes of these patients contained less protein than normal. In adrenalectomized animals there is no liberation of protein during fasting.

Adrenocorticotrophic hormone has been used in animals with lymphosarcoma. It is possible to delay the development of a transplant, but once the tumor has "taken," the progress is just as rapid as if the hormone were not given. One can produce definite temporary changes but lymphocytes regenerate very rapidly after injury and the end result can not be modified.

It seems doubtful that the protein mobilized from lymphocytes is large enough to account for the increased protein catabolism after injury.

Dr. Long, in response to the question concerning the multiplicity of adrenal actions, said that he was a unitarian. He believed that the adrenal cortex normally secretes only one hormone and that the other hormones represent the products of the biochemist rather than the efforts of the adrenal gland. There are, or course, many clinical complexities which can be rationalized by the assumption of a balance of several hormones. Perhaps tumors liberate intermediate compounds or abnormal hormones or perhaps the hormones are modified by other tissues.